FEB 29 1944

CANCER RESEARCH

Medical Library

VOLUME 4
NUMBER 3
MARCH, 1944

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

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THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CANCER RESEARCH

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Published by The International Cancer Research Foundation.

Publication Office, 1500 Greenmount Ave., Baltimore 2, Maryland.

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, $7.00. Business communications, remittances, and subscriptions should be addressed to Robert W. Briggs, Business Manager, 1500 Greenmount Ave., Baltimore 2, Md., or 2500 Lincoln-Liberty Building, Philadelphia 7, Pa.

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Entered as second class matter February 12, 1941, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 4 MARCH, 1944 NUMBER 3

Metabolic Studies in Patients with Cancer of the Gastrointestinal Tract. XIII

The Effect of Glycine on the Urinary Excretion of Creatine and Creatinine, Especially by Patients with Cancer of the Gastrointestinal Tract*

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(Received for publication September 30, 1943)

The results of a previous study from this Hospital suggest that patients with gastric cancer not only have a delayed absorption from the intestine, but also an abnormally low utilization of glycine (2). Since that amino acid is known to play a role in certain metabolic processes that probably are localized in the liver, its impaired utilization may be due to hepatic insufficiency (1).

Considerable evidence exists to favor the view that glycine is converted to guanidino-acetic acid, which then is methylated to form creatine. The first of these reactions probably takes place principally in the kidney, and the transfer of free methyl groups chiefly in the liver (4). The administration of glycine ordinarily is followed by an increased urinary excretion of creatine and creatinine (3). The investigation here reported was undertaken to ascertain whether or not the limited utilization of the amino acid by the patients is associated with a decreased formation of creatine and creatinine.

CLINICAL MATERIAL

Four groups of subjects were studied. The first of these comprised 9 adults, 3 of whom were normal, 4 had uterine fibroids, 1 had an anal ulcer, and the last a pigmented nevus. The second group, of 7 patients, was admitted to the Hospital for the treatment of benign gastrointestinal disorders; of these 6 had peptic ulcers and 1 had polyposis of the stomach. The third group consisted of 19 patient with carcinoma of the gastrointestinal tract; 1 had cancer of the esophagus, 16 of the stomach, 1 of the jejunum, and 1 of the sigmoid. The last group comprised 6 subjects with advanced cirrhosis of the liver; in 5 a prominent alcoholic history was obtained.

METHODS

All patients were fasted from 7 p.m. of the preceding night until the test was completed. At 7 a.m. on the morning of the test the bladder was emptied and urine discarded. From 7 a.m. to 10 a.m., and from 10 a.m. to 1 p.m. two complete 3 hour specimens were obtained and analyzed for their content of creatine and creatinine. At 10 a.m. 75 mgm. per kg. body weight of glycine were administered in the form of a 10 per cent aqueous solution to each subject during the course of 30 minutes.

To insure an adequate urine flow during the test period, 4 ml. of water per kg. body weight were administered at 7 a.m., and the same amount less that contained in the infusion at 10 a.m.

All subjects remained at complete bed rest from 7 p.m. of the preceding night to 1 p.m. of the test day.

Urinary creatine and creatinine were determined by the method of Folin (5).

* The authors gratefully acknowledge the grant made by the National Cancer Institute.

RESULTS

In order to be satisfied that an increased excretion of creatine and creatinine was due to the administration of the glycine and not to spontaneous fluctuations of its output, the amounts excreted by a group of 5 subjects who received no glycine were measured in two successive 3 hour urine collections. In all 5 instances the creatine and creatinine excreted during the second period had decreased (Table I).

The intravenous administration of glycine to the 9 control subjects was followed in all instances by an increased urinary excretion of both creatine and creatinine (Table II). The excess creatine output after the infusion varied from 18.2 to 89.6 mgm., and that of creatinine from 9.2 to 121.0 mgm. Likewise, greater amounts of both creatine and creatinine uniformly appeared in the urine of the 7 patients with benign gastrointestinal disorders after glycine administration. The creatine output of this group was increased by from 6.2 to 49.6 mgm., and that of creatinine by from 22.0 to 115.0 mgm. (Table III).

In contrast to these findings were the results obtained in a group of 19 patients with gastrointestinal carcinoma. After the infusion of glycine, creatine excretion rose in only 3 instances, by 5.1, 80.9, and 93.0 mgm. In the remaining 16 patients (84 per cent) the creatine output either did not significantly change or decreased by from 3.2 to 49.5 mgm. Similarly, the outputs of creatinine increased in only 3 instances by 6.5, 60.0, and 100.0 mgm., and in the remaining 16 (84 per cent) decreased by from 5.5 to 205.0 mgm. (Table IV).

TABLE I: THE URINARY EXCRETION OF CREATINE AND CREATININE BY THE SUBJECTS WHO DID NOT RECEIVE GLYCINE

		Creatine			Creatinine		
Subject	Diagnosis	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference
H.T.	Uterine fibroids	51.0	18.7	—32.3	146.0	107.0	—39.0
F.H.	Normal	27.6	12.9	—14.7	230.0	152.0	—78.0
J.S.	Pigmented nevus	18.4	6.4	—12.0	72.5	39.8	—32.7
K.L.	Cancer stomach	11.8	5.1	—6.7	112.0	57.6	—54.4
L.G.	" "	8.5	8.2	—0.3	267.0	224.2	—42.8

TABLE II: THE URINARY EXCRETION OF CREATINE AND CREATININE BY CONTROL SUBJECTS BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF GLYCINE

		Creatine			Creatinine		
Subject	Diagnosis	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference
C.S.	Normal	42.6	77.0	+34.4	108.0	239.0	+121.0
J.C.	"	23.2	49.8	+26.6	213.8	244.0	+30.2
L.D.	"	6.2	24.4	+18.2	51.2	164.0	+112.8
C.McN.	Uterine fibroids	103.7	179.0	+65.3	142.5	204.0	+61.5
H.M.	" "	41.2	110.0	+68.8	74.0	141.0	+67.0
E.L.	" "	10.4	110.0	+89.6	97.0	117.0	+20.0
H.T.	" "	16.8	36.2	+19.4	66.0	157.5	+91.5
T.S.	Anal ulcers	15.1	36.8	+21.7	106.0	116.0	+10.0
H.J.	Keloid	14.0	45.0	+31.0	131.0	140.8	+9.2

TABLE III: THE URINARY EXCRETION OF CREATINE AND CREATININE BY PATIENTS WITH BENIGN GASTROINTESTINAL DISORDERS BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF GLYCINE

		Creatine			Creatinine		
Subject	Diagnosis	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference
J.W.	Gastric ulcer	12.2	28.0	+15.8	198.0	220.0	+22.0
T.B.	" "	23.6	33.2	+9.6	78.0	193.0	+115.0
P.S.	" "	27.7	57.4	+29.7	138.0	183.0	+45.0
D.E.	" "	6.4	35.9	+29.5	84.6	126.0	+41.4
J.O'B.	" "	48.0	79.6	+31.6	96.0	180.0	+84.0
H.B.	Duodenal "	24.4	74.0	+49.6	94.0	162.0	+68.0
J.H.	Gastric polyps	4.1	10.3	+6.2	36.5	102.0	+65.5

If the patients with gastrointestinal cancer failed to excrete increased amounts of creatine and creatinine after the infusion of glycine because of their hepatic insufficiency, then a similar lack of response might be noted in patients with liver dysfunction on other bases. This, in fact, was found to be the case in 5 of 6 patients with hepatic cirrhosis. The creatine outputs remained unchanged in 1 and fell in 4 instances from acid. Only the first possibility was tested. Three grams of choline were given by mouth when the glycine again was administered to 2 patients with gastric cancer and to 2 with hepatic cirrhosis. The simultaneous administration of this source of free methyl groups (6) and the amino acid in these subjects was not followed by any increased urinary excretion of creatine or creatinine (Table VI).

TABLE IV: THE URINARY EXCRETION OF CREATINE AND CREATININE BY PATIENTS WITH GASTROINTESTINAL CANCER BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF GLYCINE

		Creatine			Creatinine		
Subject	Diagnosis	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference
K.C.	Cancer esophagus	28.0	11.5	—16.5	183.0	177.5	—5.5
H.B.	Cancer stomach	23.6	174.5	+80.9	154.4	89.0	—75.4
J.H.	" "	22.1	20.4	—1.7	120.8	93.0	—27.8
H.C.	" "	77.5	72.3	—5.2	151.0	76.0	—75.0
J.S.	" "	30.5	24.1	—6.4	188.5	158.0	—30.5
J.G.	" "	100.0	50.5	—49.5	391.0	196.0	—205.0
H.M.	" "	23.1	19.9	—3.2	82.0	182.0	+100.0
J.D.	" "	32.0	31.3	—0.7	203.0	263.0	+60.0
R.A.	" "	28.5	25.6	—2.9	306.5	192.0	—114.5
H.B.	" "	21.6	9.8	—11.8	178.0	99.5	—78.5
H.D.	" "	24.5	21.1	—3.4	270.0	177.0	—93.0
H.R.	" "	18.5	18.2	—0.3	212.0	62.0	—150.0
J.P.	" "	11.4	16.5	+5.1	92.5	82.5	—10.0
K.L.	" "	20.9	12.8	—8.1	84.0	76.5	—7.5
L.G.	" "	20.0	7.0	—13.0	138.0	120.0	—18.0
A.S.	" "	37.5	3.8	—33.7	199.5	76.5	—123.0
J.D.	" "	42.0	135.0	+93.0	151.0	138.0	—13.0
J.B.	" jejunum	48.8	40.5	—8.3	145.5	152.0	+6.5
S.W.	" sigmoid	39.4	35.6	—38.0	338.0	155.0	—183.0

TABLE V: THE URINARY EXCRETION OF CREATINE AND CREATININE BY PATIENTS WITH CIRRHOSIS OF THE LIVER BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF GLYCINE

		Creatine			Creatinine		
Subject	Diagnosis	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference
J.O'D.	Cirrhosis liver	68.0	39.1	—28.9	145.5	93.7	—51.8
E.H.	" "	29.2	21.4	—7.8	186.5	134.5	—52.0
J.M.	" "	20.7	17.4	—3.3	123.5	95.2	—28.3
E.L.	" "	3.9	31.0	+27.1	65.7	135.0	+70.3
L.S.	" "	24.5	24.0	—0.5	173.0	106.0	—67.0
J.M.	" "	56.0	49.1	—6.9	200.0	145.0	—55.0

3.3 to 28.9 mgm. In only 1 the creatine output rose by 27.1 and that of creatinine by 70.3 mgm. In the remaining 5 the creatinine output fell by from 28.3 to 67.0 mgm.

Since it appeared that patients with gastrointestinal cancer and those with cirrhosis of the liver both fail to excrete greater amounts of creatine and creatinine after the administration of glycine, the possibilities existed that this failure was due to: (a) a deficiency of the methyl groups, (b) a deficiency of the methyl acceptor—the guanidino acetic acid, or (c) an inability to transfer free methyl groups to the guanidino-acetic

DISCUSSION

From the evidence presented, the decreased utilization of glycine by patients with gastrointestinal cancer in part may be due to their impaired ability to synthesize creatine and creatinine from the amino acid. The defect does not appear to be due to a deficiency of free methyl groups, but possibly to an inability of the livers to effect the transfer. This suggestion, while consistent with the considerable hepatic dysfunction of these patients, is by no means certain, for other mechanisms reasonably could account for the failure of the glycine to increase the creatine and creatinine

output. These are: (a) That glycine was not converted by the kidney to guanidino-acetic acid. To test this hypothesis it would, of course, be necessary to administer this intermediary compound, and this has not been available for study. (b) The patients with gastrointestinal cancer may be deficient in glycine so that the amino acid administered is diverted immediately for other purposes. This explanation, however, is unlikely because of the original observation, namely, that the rate of glycine utilization by patients with gastrointestinal cancer is decreased. (c) Finally, it is possible that the patients are deficient in creatine and creatinine, and that when these substances are formed by the glycine administered, they are stored completely. Again, this explanation is improbable since the amounts of creatine and creatinine excreted by patients with cancer of the gastrointestinal tract or with hepatic cirrhosis before the administration of glycine were even greater than were those excreted by the controls.

TABLE VI: THE URINARY EXCRETION OF CREATINE AND CREATININE BEFORE AND AFTER THE ADMINISTRATION OF GLYCINE, AND OF GLYCINE AND CHOLINE CHLORIDE

Subject	Diagnosis	Supplement	Creatine			Creatinine		
			1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference	1st 3 hours' urine, mgm.	2nd 3 hours' urine, mgm.	Difference
E.H.	Cirrhosis	Glycine i.v.	29.2	21.4	—7.8	186.5	134.5	—52.0
		Glycine i.v. + Choline chloride 3 gm. p.o.	19.4	9.9	—9.5	168.0	163.5	—4.5
J.O'D.	"	Glycine i.v.	68.0	39.1	—28.9	145.5	93.7	—51.8
		Glycine i.v. + Choline chloride 3 gm. p.o.	59.0	45.5	—13.5	146.8	106.0	—40.8
J.H.	Cancer stomach	Glycine i.v.	22.1	20.4	—1.7	120.8	93.0	—27.8
		Glycine i.v. + Choline chloride 3 gm. p.o.	25.4	20.0	—5.4	142.9	113.4	—29.5
K.L.	" "	Glycine i.v.	20.9	12.8	—8.1	138.0	120.0	—18.0
		Glycine i.v. + Choline chloride 3 gm. p.o.	34.5	23.0	—11.5	117.5	110.0	—7.5

i.v. = intravenously.
p.o. = by mouth.

CONCLUSIONS

1. The intravenous administration of glycine to normal subjects and to patients with benign gastrointestinal disorders was uniformly followed by increased urinary excretion of creatine and creatinine.

2. The intravenous administration of comparable amounts of glycine to patients with gastrointestinal cancer and hepatic cirrhosis in 21 of 25 instances failed to increase their urinary output of creatine and creatinine.

3. The simultaneous ingestion of choline, as a source of free methyl groups, failed to increase the creatine and creatinine excreted in the urine of patients with gastrointestinal cancer or hepatic cirrhosis.

4. The possibility is presented that the failure of glycine to increase the output of creatine and creatinine in the urine of patients with gastrointestinal cancer is due to their coexisting hepatic insufficiency.

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The Metabolism of Pyruvate by Normal and Leukemic White Cells*

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(Received for publication September 30, 1943)

In previous investigations of the metabolism of thiamine by leukocytes (7, 1), abnormally high contents of the vitamin were found in leukemic cells. The probable explanation for this abnormality was shown to be an impaired utilization of the vitamin and not its increased ingestion, the faulty excretion, or the apparent youth of the cells involved. No explanation for the impaired utilization was advanced.

Since thiamine in the form of the pyrophosphate ester (cocarboxylase) acts as a coenzyme in some decarboxylating systems, an explanation for the impaired utilization of thiamine by leukemic cells was thought to be that: (a) thiamine did not exist as cocarboxylase in those cells, or (b) the cocarboxylase was formed but was not used because of a lack or inactivation of the enzymes involved. These possibilities now have been investigated.

CLINICAL MATERIAL

White cells were obtained for study from the following groups of persons:

1. Twenty-eight normal adults. For most experiments it was necessary to pool the cells of from 4 to 6 subjects to provide sufficient material.

2. Fourteen patients with chronic leukemia. Of these, 5 had the myeloid and 9 the lymphoid type of the disease. None were febrile.

3. Five patients with acute infections. Of these, 1 had thrombophlebitis, 1 acute pharyngitis, 1 ulcerative glossitis and septicemia, 1 had postabortal sepsis, and 1 a cellulitis of the face. All had polymorphonuclear leukocytosis.

4. Finally, marrow cells were obtained from the resected ribs of 3 patients subjected to thoracoplasty for chronic pulmonary tuberculosis.

METHODS

1. The methods used to procure the marrow and white cells have been described in previous communications (7, 1). All blood samples were obtained from subjects in a fasted state.

2. Thiamine was measured by an adaptation of the technic of Atkin, Schultz, and Frey (7).

3. Cocarboxylase was determined by the method of Goodhart and Sinclair (6), which was modified in two respects: (a) cysteine, 150 mgm. per cent was included in the medium (8), and (b) the yeast halozymase was washed with warmed (30°C) alkaline phosphate.

4. The technics used to demonstrate aerobic and anaerobic decarboxylation of pyruvate were those of Banga, Ochoa, and Peters (2), and Lohmann and Schuster (9), respectively. The latter method was modified only in that the system was adjusted to pH 7.4 with Ringer phosphate buffer.

5. Pyruvate was measured by the technic of Bueding and Wortis (5).

6. The ability of the white cells to convert pyruvate to lactate was ascertained by an adaptation of the technic described by Bueding and Goodhart (4); the conversion of pyruvate to lactate was stopped by the addition of 20 per cent trichloracetic acid.

7. Lactate was measured by the method of Barker and Summerson (3).

RESULTS

A. *Measurement of cocarboxylase.*—If the inability of the leukemic white cells to metabolize thiamine was due to the fact that the vitamin existed in an unusual form, then the ratio of cocarboxylase to total thiamine compounds in these cells would be abnormal. Accordingly, measurements of these compounds were made in 4 samples of pooled white cells each obtained from the bloods of 4 normal subjects, and in the white cells of 4 patients with leukemia. From 65 to 101 per cent of the thiamine present in *both* normal and leukemic leukocytes was found to exist in the form of cocarboxylase (Table I).

B. *Failure to demonstrate a pyruvic decarboxylase in white cells.*—Since the leukemic white cells contained normal proportions of cocarboxylase, it became

* This investigation was aided by grants from the Barbara Gillies Memorial Fund, The Jane Coffin Childs Memorial Fund for Medical Research, and the Carnegie Foundation.

necessary to ascertain whether or not the enzymes (which might require cocarboxylase for activation) were absent from the leukemic tissue.

Three samples of normal and 3 of leukemic white cells were used as a possible source of enzymes in: (a) a system in which it is possible to demonstate aerobic pyruvic decarboxylation by brain and kidney (2), and (b) a system used to manifest the activity of yeast carboxylase (9), but adjusted in these studies to pH 7.4 with Ringer-phosphate buffer. In neither system did the added pyruvate undergo decarboxylation.

Samples of the white cells of normal and leukemic individuals then were homogenized to "liberate" the enzymes, which thus might come into more intimate contact with the components of the above systems, (a) and (b). Nevertheless, pyruvate decarboxylation could not be demonstrated in these preparations.

TABLE I: THE COCARBOXYLASE CONTENT OF WHITE CELLS

Nature of white cells	Total thiamine, μgm./100 ml. cells	Cocarboxylase, μgm./100 ml. cells	Cocarboxylase/Total thiamine
Pooled normal	130	105	0.81
" "	71	60	0.84
" "	95	96	1.01
" "	183	148	0.79
L.Y. (chronic myeloid leukemia)	416	350	0.84
T.J. (chronic myeloid leukemia)	240	156	0.65
B.F. (chronic lymphatic leukemia)	280	267	0.96
P.M. (chronic lymphatic leukemia)	320	300	0.93

The possibility existed that no increased O_2 utilization or CO_2 production followed the introduction of pyruvate into the enzyme systems studied because the cells already contained sufficient amounts of the substrate. Chemical determinations of 5 specimens of normal and 5 specimens of leukemic white cells failed to reveal any pyruvate. The absence of this substance from other actively metabolizing tissue (brain and liver) likewise has been observed in this laboratory.

Therefore, if normal or leukemic white cells have enzymes analogous to the pyruvate decarboxylase of brain, kidney, or yeast, and which require cocarboxylase for their activity, it was not possible to demonstrate the existence of those enzymes by the technics used.

C. *Ability of white cells to convert pyruvate to lactate.*—Although it was not possible to demonstrate that white cells decarboxylate pyruvate, nevertheless they do utilize that substance at a considerable rate. Observations of a similar nature have been made by Beuding and Goodhart (4) on the utilization of pyruvate by red cells or whole blood.

In order to compare the ability of one sample of white cells to utilize pyruvate with that of another, it first was necessary to demonstrate that the amount of pyruvate utilized in the system studied was a function of the amount of white cells present. Accordingly, the amounts of pyruvate metabolized by different amounts of white cells obtained from 3 groups of normal persons and from 3 patients with leukemia were measured. These values, when plotted against the amounts of cells used, gave almost a linear relationship.

When this fact was established, determinations were made of the comparative ability of normal and of leukemic white cells to utilize pyruvate. Seven specimens of normal white cells, each specimen made from the pooled leukocytes obtained from the bloods of from 4 to 6 healthy subjects, metabolized from 430 to 650 μgm. of pyruvate, or an average of 510 μgm., per ml. of cells, per hour. In contrast, the white cells of 12 patients with chronic leukemia, 4 with myeloid and 8 with lymphoid disease, utilized from 800 to 2,150 μgm., or an average of 1,151 μgm., per ml. of cells, per hour. Of these 12 samples of white cells, all used more pyruvate than did any of the preparations from normal individuals (Table II).

TABLE II: THE UTILIZATION OF PYRUVATE AND THE FORMATION OF LACTATE BY THE WHITE CELLS OF NORMAL INDIVIDUALS AND OF PATIENTS WITH LEUKEMIA

White cells of		Pyruvate used/ml./hr., μgm.	Lactate formed/ml./hr., μgm.	Pyruvate lactate, per cent
Pooled normals	#1	430	490	110
	#2	520	580	110
	#3	515	620	120
	#4	650		
	#5	458	400	89
	#6	455	345	76
	#7	540	563	105
Average		510	500	102
Chronic myeloid leukemia	R.C.	1,300	924	71
	L.J.	930		
	M.F.	800	458	56
	J.L.	2,150	717	33
Chronic lymphoid leukemia	P.M.	890	282	32
	M.A.	1,000	50	5
	E.P.	900	410	46
	S.T.	1,025	650	63
	S.F.	800	889	111
	A.S.	1,109	875	79
	A.S.	1,854	37	2
	T.J.	1,060	1,020	90
Average		1,151	574	57

Since the metabolism of pyruvate by normal or leukemic white cells was accompanied by neither the apparent utilization of O_2 nor formation of CO_2, the possibility was investigated that the pyruvate merely

was reduced to lactate. Recent studies elsewhere have shown that the disappearance of pyruvate in whole blood is associated with the appearance of almost equivalent amounts of lactate (4). In 6 of the 7 experiments in which the specimens of normal white cells were studied for their ability to utilize pyruvate, the amounts of lactate that appeared during the course of the experiment were measured and were found to be equivalent to from 76 to 120 per cent (average 102) of the pyruvate that disappeared (Table II). In contrast to this finding were the relatively small proportions of lactate that appeared during the pyruvate utilization by the specimens of leukemic cells. Lactate formation by 11 of the 12 samples of leukemic white cells was found to account for from 2 to 111 per cent (average 57) of the pyruvate utilized (Table II). Of these 11 per cent values, 8 were below the lowest normal.

TABLE III: EXPERIMENTS TO DEMONSTRATE THAT LACTATE WAS DERIVED FROM PYRUVATE

Material	Lactate present before incubation, μgm.	Lactate present after one hour incubation, μgm.	Difference
1 ml. leukemic plasma and 0.135 ml. leukemic white cells	72	81.0	9.0
1 ml. leukemic plasma alone	60	68.5	8.5
Amount lactate from 1.0 ml. leukemic white cells alone			3.7
1 ml. normal plasma and 0.09 ml. normal white cells	84	88.5	4.5
1 ml. normal plasma alone	77	82.0	5.0
Amount lactate from 1.0 ml. normal white cells alone			—5.5

To establish that the lactate that appeared during the course of pyruvate utilization was formed from the pyruvate, measurements were made of the amounts of lactate formed when no pyruvate was added to the system. In two experiments it was found that normal or leukemic white cells incubated without pyruvate for 1 hour (Table III) produced no significant amounts of lactate.

The impaired ability of leukemic white cells to convert a normal proportion of pyruvate into lactate was considered possibly to be due to the apparent youth of the cells. This supposition, however, was not tenable for the following reasons:

1. The white cells of 5 patients with leukocytosis secondary to acute infections converted normal proportions of the pyruvate utilized into lactate (84 to 106 per cent). The total amounts of pyruvate used were abnormally high (Table IV) in 4 instances.

2. The immature cells obtained from the marrow of 3 patients with chronic pulmonary tuberculosis, and who had normal peripheral blood pictures, resembled normal white cells in the extent of their ability to utilize pyruvate and convert that compound to lactate. Of the 570 to 734 μgm. of pyruvate metabolized per hour by each milliliter of marrow cells, from 84 to 130 per cent was converted to lactate (Table V).

No evidence could be advanced that the reactions involving the utilization of pyruvate either by normal or leukemic white cells were dependent upon the presence of thiamine or of cocarboxylase. The addition of from 0.5 to 3.0 mgm. of thiamine or of cocarboxylase to the systems studied had no effect on the pyruvate utilization or lactate formation (Table VI). A similar lack of correlation between the ability of whole blood to utilize pyruvate and the concentration of thiamine in the medium was found by others (4). It must be emphasized that the critical experiment to show a relationship between the concentration of the vitamin and the reaction under study could not be done, namely, to remove or destroy the thiamine and cocarboxylase already present in the

TABLE IV: THE ABILITY OF WHITE CELLS OF PATIENTS WITH ACUTE INFECTIONS AND LEUKOCYTOSIS TO UTILIZE PYRUVATE AND TO FORM LACTATE

Patient	Condition	WBC	Pyruvate used/ml./hr., μgm.	Pyruvate converted to lactate, per cent
D.T.	Acute pharyngitis	22,000	487	91
J.H.	Ulcerative glossitis	24,000	2,189	91
J.K.	Thrombophlebitis	25,000	890	84
H.B.	Cellulitis of face	17,400	890	105
M.T.	Postabortal sepsis	21,000	1,755	106

TABLE V: THE ABILITY OF MARROW CELLS OBTAINED FROM PATIENTS WITH CHRONIC TUBERCULOSIS TO UTILIZE PYRUVATE AND TO FORM LACTATE

Patient	Pyruvate used/ml./hr., μgm.	Pyruvate converted to lactate, per cent
E.L.	734	114
H.R.	570	130
J.K.	700	84

TABLE VI: EFFECTS OF THIAMINE OR COCARBOXYLASE ON THE ABILITY OF WHITE CELLS TO CONVERT PYRUVATE INTO LACTATE

Cells	Component added, μgm.		Pyruvate used/ml./hr., μgm.	Lactate used/ml./hr., μgm.
Pooled normal cells		0	645	600
	Thiamine	0.5	587	608
	Thiamine	3.0	612	595
	Cocarboxylase	3.0	560	575
Leukemic cells		0	900	406
	Thiamine	0.5	1,000	525
	Thiamine	3.0	925	494
	Cocarboxylase	3.0	988	512

white cells, and then to measure the ability of those cells to convert pyruvate into lactate.

COMMENT

No explanation for the inability of leukemic white cells to metabolize thiamine normally can be advanced at this time. It would appear that those cells do contain normal proportions of cocarboxylase, but the existence of enzyme systems known to require that compound as a coenzyme has not been demonstrated either in normal or neoplastic white cells. Pyruvate, nevertheless, is utilized by the white cells, but that utilization probably does not require O_2 directly, nor is CO_2 produced. Rather, it appears that normal white cells, like erythrocytes or whole blood (4), reduce the pyruvate quantitatively to lactate.

In contrast, it has been found that leukemic cells not only utilize more pyruvate than do normal white cells, but also convert abnormally small proportions of the compound to lactate. What other substances are formed from the pyruvate used by the leukemic cells is not known.

CONCLUSIONS

1. From 65 to 101 per cent of the thiamine present both in normal and leukemic white cells exists in the form of cocarboxylase. Nevertheless, those enzyme systems that are known to utilize cocarboxylase as a coenzyme could not be demonstrated in either the normal or leukemic cells.

2. Qualitative and quantitative differences have been observed for the utilization of pyruvate by normal and leukemic white cells. The normal cells apparently utilize less pyruvate and, in most instances, convert a greater proportion of that compound to lactate than do the leukemic cells. This abnormality of the neoplastic cells probably is not due simply to their apparent youth.

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The Carcinogenicity of *p*-Dimethylaminoazobenzene in Diets Containing Hydrogenated Coconut Oil*†

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(Received for publication September 22, 1943)

The original observation that the carcinogenic potency of *p*-dimethylaminoazobenzene varies with diet (19) has been followed by numerous studies of individual dietary substances on the development of hepatic tumors with this agent. Much emphasis has been placed on the vitamins of the B complex (4, 9, 17) or on certain of these vitamins plus proteins or amino acids (5, 9, 16, 30). Comparatively little attention has been given to the fats, although at least three fatty extracts are reported to reduce the carcinogenic effectiveness of the azo dye when fed with it at levels of 5 per cent of the diet or less: rice bran oil (28), an ether extract of yeast (28), and a similar extract of liver (16). However, the original materials from which these extracts were prepared all happen to be anticarcinogenic by virtue of nonfatty substances such as proteins and B vitamins that they contain. Analyses of rice bran oil for riboflavin and other B vitamins revealed the presence of many water-soluble materials (28), although in amounts that were probably insufficient to account for the entire anticarcinogenic effect observed.

A second group of fats appears to have no significant effect on the development of hepatic tumors due to *p*-dimethylaminoazobenzene. These include cod liver oil at 1 or 2 per cent of the diet (16); olive oil at 3 per cent (10, 19); cottonseed oil (16), corn oil (9, 16), butter fat (16), or wheat germ oil (27) at 5 per cent; and partially hydrogenated vegetable oil (primex) at 20 or 30 per cent of the diet (16). These latter oils, however, were usually fed in experiments designed to reveal the effects of the nonfatty constituents of the diet, and true differences between the various oils may therefore have been masked. Another complication arises from the fact that as the percentage of fat in the diet increases, the amount of food, and therefore of carcinogen consumed by the animal, decreases. Nevertheless, these "nonprotective" fats represent a considerable range in such chemical characteristics as degree of unsaturation, saponification equivalent, and autooxidizability.

György and his associates (7) compared the carcinogenic effectiveness of diets low in protein and high in fat and reported that crisco and butter fat were cocarcinogenic as compared to lard. As an explanation of the protective effect of the lard diet a destruction of the carcinogen *in vitro* was assumed.

The present study deals with the effect of hydrogenated coconut oil on the incidence of liver tumors in rats fed *p*-dimethylaminoazobenzene in synthetic diets. From a chemical point of view this oil represented a considerable departure from the corn oil previously employed for the production of liver tumors in rats fed synthetic diets (17). The hydrogenated coconut oil contained no unsaturated fatty acids, whereas the corn oil was highly unsaturated, linoleic acid constituting approximately 42 per cent of the fatty acids present; the hydrogenated coconut oil was low in antioxidants whereas the corn oil contained appreciable amounts of tocopherol; and, furthermore, most of the fatty acids in the hydrogenated coconut oil were of relatively short chain length. Another reason for the comparison of this particular pair of oils arose from the observation that the formation of hepatic (17) and other (11) types of tumors is diminished on diets low in pyridoxine. In multiple deficient diets pyridoxine and the essential fatty acids appear able to replace one another to a certain extent, for either by itself is sufficient to alleviate most of the symptoms of acute acrodynia (23, 26). The possibility existed that a similar association of these two factors might manifest itself in the formation of hepatic tumors.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† This investigation was aided by grants from the Jonathan Bowman Fund for Cancer Research and from the Wisconsin Alumni Research Foundation. Two of us, B. E. Kline, prior to July, 1943, and J. A. Miller, thereafter, are indebted to the Finney-Howell Foundation for financial support. A gift of crystalline B vitamins from Dr. D. F. Robertson of Merck and Company is gratefully acknowledged. Thanks are due also to Dr. T. M. Godfrey of Lever Bros. Company for the hydrogenated coconut oil used in these studies, and to our colleague, Mr. D. L. Miner, who assisted in some of the initial experiments herein described.

METHODS

As in our previous studies (17), young, mature, albino Sprague-Dawley rats 160 to 190 gm. in weight were fed 0.06 per cent of *p*-dimethylaminoazobenzene for a period of 120 days. The animals were kept in groups of 7 and 8 in screen-bottomed cages; food and water were given *ad libitum*. The dye was incorporated in the rations by dissolving it with heat in the fat of the diets. The rations were mixed in amounts sufficient for 2 to 4 weeks and stored at 0° to 5° C. After 120 days the livers were examined by laparotomy and the animals continued for an additional 2 months on the same diet as before, but minus the azo dye. At 6 months a final examination of the livers was made. The diets fed had the following composition:

Casein (vitamin-free [1])	120
Cerelose [2]	800 or 790
Corn oil or hydrogenated coconut oil	40 or 50
Salts	40
p-Dimethylaminoazobenzene	0.6

[1] Crude casein was washed in cloth bags at room temperature for 5 days with 15 changes of tap water and 2 changes of distilled water (20 gallons for each 10 pounds). After being dried and ground it was finally extracted at 50° C. for 4 days with two changes of ethyl alcohol (4 gallons for each 10 pounds). By the methods indicated each gram of the purified casein contained an average of 0.6 μgm. thiamin (thiochrome), 0.6 μgm. riboflavin (fluorometric), less than 1 μgm. pantothenic acid (growth of *Lactobacillus casei*, determination made by Dr. A. L. Neal), and 0.17 μgm. pyridoxine (growth of *Saccharomyces carlsbergensis*, determination made by Mrs. E. C. Miller).

[2] A pure glucose monohydrate obtained from Corn Products Refining Company.

Each rat received one drop of halibut liver oil weekly. Crystalline vitamin B supplements added per kilogram of ration were:

Thiamin chloride	3.0 mgm.
Riboflavin	2.0 mgm.
Calcium pantothenate	7.0 mgm.
Pyridoxine hydrochloride	0.0, 0.2, or 2.5 mgm.
Choline chloride	30.0 mgm.

The hydrogenated coconut oil used in these experiments was a refined oil, hydrogenated in the presence of nickel at 140° C., and deodorized at 180° C. for 5 hours at a pressure of 5 mm. Hg. It was a pasty solid at room temperature and had a melting point of 36.0° to 36.2° C. (Wiley). The iodine numbers of the two lots of oil used for the two series of experiments reported below were 0.33 and 0.40 respectively. No tocopherol was found to be present as determined by a modification of the Furter-Meyer test (22).

Two of the groups receiving the hydrogenated coconut oil were also fed daily supplements of 2 drops (40 mgm.) of ethyl linolate given by dropper. This amount of linoleic acid is much less than that consumed by the animals receiving diets containing 5 per cent of corn oil, but is sufficient for the growth and general well-being of young rats on a fat-free diet (15). The ethyl linolate was prepared according to a modification of the method of Rollet (24). Crystalline 9,10,12,13-tetrabromostearic acid, m.p., 115° C., was isolated from the brominated fatty acids of corn oil, decomposed with zinc in absolute ethanol-HCl, and the resulting ethyl linolate freed of fatty acid and dried. The ester was not redistilled but was sealed *in vacuo* in 5 gm. lots containing 10 μgm. of synthetic α-tocopherol per gm. Each 5 gm. tube supplied 30 animals for 4 days and was kept in the refrigerator after opening.

The various combinations of corn oil, hydrogenated coconut oil, pyridoxine, and ethyl linolate fed are listed in Table I.

RESULTS

Most of the rats on the various diets maintained their weights well, their general physical condition remained good, and the survival at 4 months was 100 per cent in 5 of the 8 groups (Table I). In the initial series (Table I, groups I to III) a normal tumor production of 53 per cent at 6 months was observed in a diet containing 5 per cent of corn oil and 2.5 mgm. of pyridoxine per kg. of ration. This agrees with previous experience with this diet (17). However, when the corn oil was replaced by 4 per cent of hydrogenated coconut oil either in the presence of 2.5 mgm. of pyridoxine per kg. of diet (group II) or in the absence of added pyridoxine (group III) the animals were almost completely protected against the formation of tumors. Only one animal fed the hydrogenated coconut oil developed a tumor by 6 months, and the livers of the others were nearly normal in appearance. The protective effect of the hydrogenated coconut oil could not be ascribed either to a low caloric intake or to a reduced consumption of carcinogen, for the average daily food consumption in the groups getting hydrogenated coconut oil was as high as, or higher than, that in the control groups getting corn oil.

In a second series (Table I, groups IV to VIII) both corn oil and hydrogenated coconut oil were fed at a level of 5 per cent. The pyridoxine levels were 2.5 and 0.2 mgm. per kg. of ration. In addition ethyl linolate (stabilized with a small amount of synthetic α-tocopherol) was given orally at the level of 40 mgm. per rat daily to one of each of the two groups receiving medium and low amounts of pyridoxine. At 4 months a tumor incidence of 36 per cent was observed in rats fed the corn oil control diet. No tumors were found in any of the rats fed the hydrogenated coconut oil diets irrespective of the levels of pyridoxine or ethyl linolate given. At 4 months the groups fed hydrogenated coconut oil were subdivided; in each group 10 rats continued to receive the dye for the

following 2 months while the remaining 5 rats were fed the dye-free diet as usual. At the six months examination the tumor incidence in the group fed corn oil had risen to 64 per cent while only 2 tumors were found in the four groups that received the dye continuously in diets containing hydrogenated coconut oil. No tumors were observed in the four groups that were given the dye for only 4 months in similar diets; in other words, the hydrogenated coconut oil again exerted a pronounced anticarcinogenic effect. As in the previous series the livers of the protected rats were nearly normal in appearance. The survival was good and the consumption of azo dye was comparable with that of the control group.

linoleic acid . . ." When the latter substance, which was toxic to rats even in the absence of carcinogen, was incorporated in the diet at a level of 16 per cent, a visible destruction of the dye occurred *in vitro* within 5 days. No destruction of the dye was observed in the lard diet, which had proved to be noncarcinogenic, but it was nevertheless "assumed that the liberation of unsaturated fatty acids in the process of digestion opens the way to the decomposition of butter yellow before it reaches the liver cells."

Since there may be considerable discrepancy between the fate of the dye in whole fats in the digestive tract, as contrasted to its behavior in a free acid *in vitro,* it appeared desirable to study the stability of *p*-dimethyl-

TABLE I: INCIDENCE OF HEPATIC TUMORS IN RATS FED CORN OIL OR HYDROGENATED COCONUT OIL

Group *	Fat, %	Pyridoxine hydrochloride, μgm. per 10 gm. diet	Ethyl linolate, mgm. per rat per day	Average starting weight, gm.	Average weight at 4 months, gm.	Average food consumption, gm. per rat per day	Survival † at		Tumors ‡ at			Cirrhosis
							4 mo.	6 mo.	4 months	6 months		
										dye for 4 mo.	dye for 6 mo.	
I	5 corn oil	25	0	161	166	7.5	15/15	—	2/15	8/15	—	Moderate-severe
II	5 HCNO §	25	0	166	190	9.3	13/15	—	0/13	1/13	—	None or mild
III	5 HCNO	0	0	181	169	7.5	12/15	—	0/12	0/12	—	None or mild
IV	5 corn oil	25	0	191	193	8.0	11/15	—	4/11	7/11	—	Moderate-severe
V	5 HCNO	25	0	185	192	9.8	15/15	12/15	0/15	0/5	1/7	None or mild
VI	5 HCNO	25	40	183	183	10.8	15/15	13/15	0/15	0/5	0/8	None or mild
VII	5 HCNO	2	0	166	157	7.5	15/15	15/15	0/15	0/5	0/10	None
VIII	5 HCNO	2	40	170	159	7.7	15/15	12/15	0/15	0/4	1/8	None or mild

* Groups I to III constitute one series of experiments and are directly comparable; groups IV to VIII comprise a second series of experiments.

† Survival = Number living over number at start.

‡ Tumors = Number with tumors over number surviving at 4 or 6 months.

§ HCNO = Hydrogenated coconut oil.

The stability of p-dimethylaminoazobenzene in the diet and in the gastrointestinal tract.—It has been pointed out previously that the protective effect of diet against the action of *p*-dimethylaminoazobenzene might be exerted through several quite different channels (17) and György and his co-workers (7) placed particular emphasis on the conservation and destruction of the azo dye *in vitro,* before it gets a chance to act in the critical carcinogenic areas. Obviously such protection of the animal would merely be a reflection of a reduced intake of effective carcinogen. A more basic question is whether the observed dietary modification of the effect of *p*-dimethylaminoazobenzene is a cellular process. György and his group fed the dye in rations containing only 6 per cent of casein, and the B vitamins were given as separate supplements. When the diet contained 23 per cent of lard, liver tumors failed to develop, while the presence of crisco or butter fat in the diet "proved to be procarcinogenic." The explanation of this effect was stated as follows: "The answer has been provided by results of still other experiments in which the source of fat was . . .

aminoazobenzene in a series of selected diets known to vary widely in carcinogenicity. For this purpose an accurate and specific method for the determination of *p*-dimethylaminoazobenzene was devised. The dye readily dissolves in strong hydrochloric acid to give a red solution having an absorption maximum at 516 mμ. The red complex is insoluble in ethyl ether and petroleum ether but can be extracted from aqueous solutions with chloroform. Furthermore the intensity of the red color in acid solution is considerably greater than the corresponding yellow color in ordinary organic solvents.

The procedure finally adopted for the analysis of the diets was as follows: 1 gm. of food (approximately 0.6 mgm. azo dye) was extracted with several 5 to 10 cc. portions of petroleum ether and the extracts filtered and made up to 50 cc. In highly rancid diets difficulty was experienced in obtaining complete extraction of the dye as tested by adding 1:1 HCl to the residue. In such cases acetone was used to complete the extraction and was then washed out of the petroleum ether with water. Five cubic centimeters of the combined

extracts were placed in a separatory funnel and extracted twice with 10 cc. portions of 1:1 hydrochloric acid. The acid extracts were made up to a volume of 30 cc. (approximately 60 μgm. azo dye) with more acid and the concentration, C, of the dye ascertained by measuring the density, L, of this final solution through the 520 mμ. filter of an Evelyn photoelectric macrocolorimeter. The colorimeter constant, K, in the relation $C=KL$ was found to be 2.98 μgm. dye/cc. With this procedure it was easily possible to measure as little as 1 μgm. of *p*-dimethylaminoazobenzene in 10 cc. of acid with an accuracy of ± 5 per cent.

coconut oil, but also those containing corn oil in the presence of rice bran concentrate or of mixtures of synthetic B vitamins in amounts somewhat different from those in the control diet (Table II).

There does not appear to be any reason to attribute antioxidant properties to different mixtures of crystalline B vitamins (6). Apparently the experimental procedure of spreading corn oil on a dry diet and exposing it to air at room temperature for 90 days was just sufficient to destroy the antioxidants in a certain percentage of samples, and once autoxidation began, it proceeded rapidly. Should the stabilization of the azo

TABLE II: STABILITY OF *p*-DIMETHYLAMINOAZOBENZENE IN VARIOUS DIETS *In Vitro* AFTER STORAGE IN THE DARK AT DIFFERENT TEMPERATURES

Diet †	Fat, %	Initial azo dye content, %	% change in azo dye content from initial level * after							
			15 days at		30 days at		60 days at		90 days at	
			−5°	25°	−5°	25°	−5°	25°	−5°	25°
Synthetic control	Corn oil 5	0.059	+1.7	−5.1	+6.8	+6.8	—	—	+5.1	−75 (rancid)
2% Rice bran extract ‡ crude casein	Corn oil 5	0.056	+5.4	0.0	+12.5	+7.2	—	—	−3.6	+1.8
HCNO § 2 μgm. pyridoxine	HCNO 5	0.060	+5.0	0.0	+6.7	0.0	—	—	+8.3	+8.3
HCNO	HCNO 5	0.062	+9.7	0.0	0.0	+3.2	—	—	−4.8	−1.6
2 μgm. Pyridoxine, 50 μgm. flavin	Corn oil 5	0.054	0.0	+1.8	+7.4	0.0	+3.7	+1.8	—	—
0.035% Niacin	Corn oil 5	0.055	0.0	+1.8	+3.6	−1.8	0.0	0.0	—	—
Egg white	Corn oil 5	0.057	−5.3	0.0	−1.8	−3.5	−7.0	0.0	—	—

* Except for the change observed in the synthetic control diet at 90 days at 25°, the variations can be ascribed to sampling and to changes in the moisture content of the diets.

† These diets consisted of 12 per cent protein, usually purified casein, and 4 per cent salts, with cerelose as the remaining major constituent. B vitamins included 25 μgm. pyridoxine hydrochloride, 30 μgm. thiamin chloride, 20 μgm. riboflavin, 70 μgm. calcium pantothenate, and 300 μgm. choline chloride per 10 gm. diet unless otherwise noted.

‡ "Vitab Rice Bran Concentrate," National Oil Products Co., Harrison, N. J. No crystalline vitamins were added to this diet.

§ HCNO = Hydrogenated coconut oil.

The diets analyzed included the synthetic diets containing corn oil or hydrogenated coconut oil as described in the present paper, and a diet containing 2 per cent of rice bran concentrate and 12 per cent of crude casein, selected because of the unusually high carcinogenicity of this particular combination (16). Other diets were high in riboflavin and low in pyridoxine, high in niacin, or contained egg white as the source of protein. The freshly mixed diets were stored in the dark at room temperature (25° C. ±) and at −5° C. and analyzed periodically.

The azo dye was found to be very stable in the various diets *in vitro*. An appreciable destruction of the dye was observed in only one sample, the synthetic diet containing corn oil stored at room temperature for 90 days (Table II). This sample had developed a very strong rancid odor. Neither destruction of the dye nor rancidity were observed when the same diet was stored at −5° for 90 days, nor was any loss of the dye detected in any of the other diets. The stable diets included not only those containing hydrogenated

dye prove to be essentially a matter of preventing rancidity, the stability observed in the presence of the rice bran concentrate could be attributed to the antioxidants in the concentrate (6, 7).

Nevertheless, it is apparent that there was no correlation between the stability of the carcinogen in prolonged experiments *in vitro,* and the number of tumors that developed when the various diets were fed to rats. Tumors failed to develop on the diets containing hydrogenated coconut oil, in which the dye was perfectly stable, whereas a high incidence of tumors (50 to 66 per cent) has been observed consistently on the one diet in which the dye failed to survive the drastic conditions imposed *in vitro*. It should be pointed out that in the feeding experiments the diets were routinely stored at −5° C., at which temperature the carcinogen was stable in all the rations. The exposure of diets to room temperature prior to feeding never exceeded one week, whereas even in the control diet the azo dye was stable for at least 30 days at this temperature. Thus it appears most unlikely

that the variations in tumor incidence observed with the different diets were due to a destruction of carcinogen prior to the consumption of the diet by the animals.

Another possibility considered is that part of the azo dye was destroyed in the gastrointestinal tract, and that more destruction occurred in the presence of hydrogenated coconut oil than in corn oil. This possibility, however, does not readily lend itself to experimentation. Measurements can of course be made of the amount of dye disappearing from the digestive tract when the two oils are fed but much of the loss, if not all, represents dye that has been absorbed by the animal. Furthermore, the proportion of dye recovered in the tissues is very small (10). When a fasted rat is fed a few grams of the synthetic diet containing *p*-dimethylaminoazobenzene, the gastric contents appear yellow except for isolated pockets of red near the stomach wall, and virtually all the dye can be recovered from the stomach. Thereafter the amount of dye in the stomach decreases, and small amounts appear in the small intestine. But this latter amount is never large, and even 12 to 24 hours after the ingestion most of the dye still remains in the stomach. Frequently the amount of dye in the small intestine is too small to be measured accurately. Furthermore, a yellow substance is present that turns red in the presence of strong acid, and therefore presumably is related to the original dye, but that differs from the dye itself in that neither the yellow form nor the red is extracted from water with chloroform.

Preliminary results indicated that somewhat more color was present in the lower third of the small intestine when the *p*-dimethylaminoazobenzene was fed in hydrogenated coconut oil than when it was fed in corn oil. The amount remaining in each case, however, was such a small fraction of the total pigment ingested that no conclusions could be drawn as to either the stability or absorbability of the dye from the tract in the presence of these two oils.

DISCUSSION

The striking differences observed in the carcinogenic potency of *p*-dimethylaminoazobenzene with corn oil and hydrogenated coconut oil respectively might be explained in a number of ways. Corn oil might contain a cocarcinogenic ingredient that is lacking in the hydrogenated coconut oil; the coconut oil might contain an anticarcinogenic factor that is lacking in the corn oil; or suitable combinations of cocarcinogenic and anticarcinogenic factors in the two oils might be involved. Present data do not permit identification of the chemical responsible for the great difference in carcinogenicity observed on the two diets, for the oils differ in many particulars such as chain length, unsaturation, and antioxidants, and any of these, or all, might prove to be significant. The experiments *in vitro* seem to indicate that with these two oils equal amounts of dye are consumed per gram of ration, and since the total amount of food consumed by the rats on the two diets was the same, it would follow that the animals in both groups were ingesting the same amount of carcinogen.

Experiments on the disappearance of dye from the gastrointestinal tract failed to reveal any appreciable difference between the two diets. These experiments were obviously inconclusive, yet in the absence of any supporting evidence there appears to be little reason to impute the anticarcinogenic effect of hydrogenated coconut oil to a reaction within the digestive tract. Accordingly, the different incidences of tumors on the two oils are attributed to reactions within the animal itself.

Fats have previously been reported to influence the development of tumors in two quite different types of experiment. In the first type diets high in fat accelerated the production of skin tumors due to ultraviolet light (2) or to the local application of carcinogenic hydrocarbons (1). Fat also increased the total number of tumors that appeared (12, 29). However, in contrast to the present results with hepatic tumors, all types of fat appeared to be effective in promoting the development of the skin tumors (8). With these latter tumors the action of the fat was partly local (25), presumably resulting in a better penetration of hydrocarbon into the skin. Furthermore, the increased number of tumors observed appeared to be associated with an increased consumption of calories by the animals fed the high-fat diets (13).

Another effect of fat on tumor formation has been that the carcinogenicity of hydrocarbons injected subcutaneously depends upon the nature of the fat in which the carcinogen is dissolved. For example, when dibenzanthracene was injected into fowls in lard solution, many tumors developed, but when the hydrocarbon was administered in egg-yolk fat or in chicken fat, no tumors appeared (20). Benzpyrene, injected subcutaneously into mice, was much more carcinogenic when dissolved in olive oil, in sesame oil, or in arachis oil than when injected in mouse fat, in organic solvents, or as a fine powder (3, 18, 21). The literature has recently been summarized by Dickens and Weil-Malherbe (3). In a very extensive series of experiments Leiter and Shear (14) demonstrated that benzpyrene was much less effective when injected in "lard residue," the portion solid at 38°, than when injected in "lard filtrate," the portion liquid at 38°. Fractionation studies pointed toward the saturated glycerides of high molecular weight as the components responsible for the retarding action.

There is some parallel between the experiments with hydrocarbons in various oils and the experiments with *p*-dimethylaminoazobenzene described in the present paper, because in both types of experiments the essential differences in carcinogenicity depended upon the

nature of the oil used. However, the chemical factors responsible in the two phenomena may prove to be quite dissimilar, since the azo dye differs considerably from the hydrocarbons in its chemical behavior, and somewhat different methods of producing tumors with these respective carcinogens are employed. It is possible that the observed effects of the oils are due to their relation to a specific carcinogen as, *e.g.*, to the rate of diffusion of the hydrocarbon out of an injected oil globule, rather than to the fundamental carcinogenic reaction itself. The effect of hydrogenated coconut oil on hepatic tumors may likewise be mainly on the metabolic fate of the specific azo dye involved.

SUMMARY

When rats were fed *p*-dimethylaminoazobenzene in a synthetic diet containing corn oil, the incidence of hepatic tumors at 6 months ranged from 53 to 64 per cent. However, when the corn oil was replaced by hydrogenated coconut oil the tumor incidence never exceeded 8 per cent, while in most groups it was zero. This effect of the hydrogenated coconut oil persisted when the rats received 25 μgm. of pyridoxine and/or 40 mgm. of ethyl linolate daily.

By means of an analytical method adequate for the quantitative determination of 1 μgm. of *p*-dimethylaminoazobenzene, it was demonstrated that the azo dye was very stable in the diets *in vitro*. Attempts to demonstrate differences in the stability of the dye in the digestive tract were unsuccessful. It therefore appeared that the differences in carcinogenicity observed in the two oils were due to changes within the animal itself.

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Observations on the Inherited Susceptibility to Spontaneous Mammary Cancer in Mice*

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(Received for publication November 29, 1943)

Many experiments on the development of spontaneous mammary cancer in mice have demonstrated that an inherited susceptibility plays an important role, but no one has yet ascertained what the make-up of the character may be. Whenever inbred strains have been tested the results in the hybrid generations have been in accord with the genetic theory that the susceptibility is transmitted as a dominant.

In addition to the inherited susceptibility, other inciting influences operate in the genesis of this type of neoplasia. These are the mammary tumor milk influence and the estrogenic and associated hormones (7).

Data have been presented on the incidence of mammary cancer in several generations of hybrid mice derived by mating animals of a high cancerous and a low cancerous strain (6, 7, 12). Previous reports have included only preliminary observations on mice of four backcross generations to the low cancerous stock. The results are now complete.

MATERIAL AND METHOD

The inbred A and C57 black (B) strains were used as the parental stocks. Spontaneous mammary carcinoma developed in approximately 95 per cent of the breeding females of the A stock (10), and in 1 per cent of those of the B stock (7).

Reciprocal matings were made between mice of the two stocks to produce the first generation hybrids and these hybrids were mated *inter se* to give the mice of the following generations. In designating the cross, the maternal parent is always given first, as, A♀ × B♂ = ABF_1 (Table I). Mice of the inbred strains and the hybrid generations were either nursed by their mothers or were fostered by females of the reciprocal strain. When BAF_1 mice were nursed by their maternal parents of the B stock, they were called BAF_1 by B; when they were transferred soon after birth to females of the A stock, they were called BAF_1 by A. Hybrids of the subsequent generations were always nursed by their mothers, but they are designated by the strain that nursed the mice of the first hybrid generation. Because of the difficulty in removing the young born to females of cancerous strains before they had suckled, mice of the ABF_1 by B generation were obtained as follows. The young cast by females of the cancerous A strain were transferred a few hours after birth to lactating females of the B stock. These fostered mice were used as breeders and the progeny or descendants of noncancerous females were mated to males of the B stock. The resulting hybrids were termed ABF_1 by B.[1] Thus the milk agent was eliminated before the females of the A stock were mated.

Mice of the backcross generations to the B stock were produced by mating F_1 or F_2 females to males of that strain. The hybrids were called BBC (B backcross). The maternal parents are represented in the text by the prefix before BBC.

The mice were continued as normal breeding females; that is, they were permitted to nurse their progeny. Generally the females were removed from the breeding pens before their litters were born and were returned after their young had been weaned.

To produce the experimental animals it was possible either to use a large number of mothers and obtain a few young from each one, or to mate a small number of mothers and secure as many progeny as possible from each. The latter method was selected, although it required a longer time to acquire the mice and consequently delayed the final results. Because of the large number of animals included in the study, it was impossible to have all under observation at one time. However, mice of reciprocal generations were studied during the same period.

The mice received Purina fox chow and an unlimited amount of water.

Only spontaneous mammary carcinomas were tabulated.

* These experiments were started at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and were assisted by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The National Cancer Institute.

[1] ABF_1 hybrids born to females of the cancerous A stock have been fostered by females of the B stock. Their incidence of mammary cancer was 8 per cent (8).

RESULTS

Table I gives the number of mice observed in each stock or hybrid generation, the nursing mothers, how the hybrids were produced, and the incidence of mammary cancer. The cancerous and noncancerous ages are expressed in days.

The breeding females of the cancerous A stock, born during the same interval as the hybrid animals, had an incidence of 95.1 per cent, and the mice of the fostered group of the same stock (A by B) gave 6.1 per cent. The noncancerous fostered mice survived approximately 120 days beyond the average cancer age of per cent. The average noncancer age exceeded 600 days. Approximately 600 F_1 mice were studied.

The F_2 by A group consisted of 391 animals, 277 ABF_2 by A (77.3 per cent cancerous) and 114 BAF_2 by A (69.3 per cent cancerous). The difference between the incidences was 8.0 per cent (1.65 × S.E.). The respective cancer ages were 398 and 452 days and the noncancer ages 535 and 632 days. The hybrids descended from mothers nursed by females of the low cancerous stock gave incidences of 0.5 per cent (BAF_2 by B, 401 mice) and 1.9 per cent (ABF_2 by B, 152 mice).

TABLE I: INCIDENCE OF SPONTANEOUS MAMMARY CARCINOMA IN FOSTERED AND NONFOSTERED FEMALES OF THE A AND B (C57 BLACK) STOCKS AND THEIR HYBRIDS. ALL WERE USED AS BREEDERS

Stock	Nursed by	Mating	No.	% Ca.	Average age in days	
					Cancer	Noncancer
A	A	A♀ × A♂	972	95.1%	286	426
ABF_1 by A	A	A♀ × B♂	123	94.4%	336	369
ABF_2 by A	ABF_1 by A	ABF_1♀ × ABF_1♂	277	77.3%	397	535
ABF_3 by A	ABF_2 by A	ABF_2♀ × ABF_2♂	339	67.0%	378	545
ABF_1—BBC	ABF_1 by A	ABF_1♀ × B♂	311	60.1%	426	626
ABF_2—BBC	ABF_2 by A	ABF_2♀ × B♂	283	50.5%	436	669
B	A	B♀ × B♂	98	18.4%	420	518
BAF_1 by A	A	B♀ × A♂	111	90.1%	346	568
BAF_2 by A	BAF_1 by A	BAF_1♀ × BAF_1♂	114	69.3%	452	632
BAF_3 by A	BAF_2 by A	BAF_2♀ × BAF_2♂	278	65.8%	388	577
B	B	B♀ × B♂	275	0.0%	—	489
BAF_1 by B	B	B♀ × A♂	212	0.5%	435	640
BAF_2 by B	BAF_1 by B	BAF_1♀ × BAF_1♂	401	0.5%	560	639
BAF_3 by B	BAF_2 by B	BAF_2♀ × BAF_2♂	228	0.9%	570	633
BAF_1—BBC	BAF_1 by B	BAF_1♀ × B♂	224	0.4%	526	653
BAF_2—BBC	BAF_2 by B	BAF_2♀ × B♂	267	0.7%	585	653
A	B or CBA	A♀ × A♂	525	6.1%	378	504
ABF_1 by B	Fostered A	Fost. A♀ × B♂	148	1.4%	375	651
ABF_2 by B	ABF_1 by B	ABF_1♀ × ABF_1♂	152	1.9%	555	657
ABF_3 by B	ABF_2 by B	ABF_2♀ × ABF_2♂	183	1.1%	525	639
		Total	5,521			

those of the control, or unfostered, group. The fostered mice were descended from females nursed by mothers of the B or CBA low cancerous strains.

None of the breeding females of the B stock nursed by their maternal parents developed mammary cancer, but 18.4 per cent of the mice suckled by females of the A stock gave rise to that type of neoplasm. Mice from several sublines of the B stock were probably included in each series.

In the F_1 population, the ABF_1 by A hybrids gave an incidence of 94.4 per cent and the mice of the BAF_1 by A generation an incidence of 90.1 per cent. The respective average cancer ages were 336 and 346 days. The degree of significance between the ratios was 1.21 times the standard error (S.E.). The BAF_1 by B mice showed growths in 0.5 per cent of the total and the animals of the ABF_1 by B group had an incidence of 1.4

Mammary cancer developed in 67.0 per cent of the ABF_3 by A (378 days) and in 65.8 per cent of the BAF_3 by A animals (388 days). The reciprocally nursed mice gave incidences of 1.1 per cent and 0.9 per cent. Over 1,000 F_3 mice were observed.

Observations on the ABF_1 by A—BBC mice resulted in an incidence of 60.1 per cent at an average cancer age of 426 days; the incidence in BAF_1 by B—BBC hybrids was 0.4 per cent. When ABF_2 by A females were mated to males of the B stock the progeny (ABF_2 by A—BBC) showed tumors in 50.5 per cent at an average age of 436 days. Mice of the reciprocal generations, BAF_2 by B—BBC, had an incidence of 0.7 per cent.

Because no significant variation was observed in the incidence of mammary cancer in the reciprocal hybrids nursed by females of the cancerous A stock, the data have been combined and presented in Table II.

The incidences for F_1 by A and F_2 by A hybrids were 92.3 per cent and 74.9 per cent respectively,[2] the cancer ages were 341 and 612 days.

Mice of the F_3 by A generations were descended either from cancerous or noncancerous F_2 mothers. Offspring of cancerous mothers gave an incidence of 70.9 per cent and the progeny of noncancerous mothers had an incidence of 45.9 per cent. The difference in the incidences was 25.0 per cent (5.02 × S.E.). The incidence for the total, 617 mice, was 66.5 per cent.

Only cancerous mothers were used to produce the ABF_1 by A—BBC hybrids, and 60.1 per cent of the progeny died cancerous. The animals of the ABF_2 by A—BBC generation had either cancerous or noncancerous mothers. The progeny of the former were observed to have an incidence of 62.8 per cent and those with noncancerous mothers an incidence of 5.0 per cent.

The hybrids nursed by females of the B stock have not been grouped in Table II since the incidence did not exceed 2 per cent in any group.

TABLE II: DATA FOR THE TOTAL NUMBER OF RECIPROCAL HYBRIDS NURSED BY FEMALES OF THE A STOCK. THE OBSERVATIONS FOR THE F_3 AND ABF_2—BBC HYBRIDS ARE TABULATED BY CANCEROUS AND NONCANCEROUS MOTHERS. ALL OTHERS HAD CANCEROUS MOTHERS. THE DIFFERENCE BETWEEN THE INCIDENCES FOR THE TOTAL NUMBER OF F_1 BY A AND A BY A MICE WAS 2.74% ± 1.50 OR 1.83 × S.E.

Stock	No.	+	−	% Ca.	Average age in days		Standard error of difference
					Ca.	Nonca.	
ABF_1 by A	123	116	7	94.4%	336	369	4.22% ± 3.49 or 1.21 × S.E.
BAF_1 by A	111	100	11	90.1%	346	568	
Total F_1 by A	234	216	18	92.3%	341	490	
ABF_2 by A	277	214	63	77.3%	397	535	7.96% ± 4.82 or 1.65 × S.E.
BAF_2 by A	114	79	35	69.3%	452	632	
Total F_2 by A	391	293	98	74.9%	412	570	
ABF_3 by A	339	227	112	67.0%	378	545	1.13% ± 3.82 or 0.30 × S.E.
BAF_3 by A	278	183	95	65.8%	388	577	
Total F_3 by A:							
Cancer F_2 mothers	508	360	148	70.9%	380	557	25.00% ± 4.98 or 5.02 × S.E.
Nonca. F_2 mothers	109	50	59	45.9%	401	565	
Total	617	410	207	66.5%	383	559	
ABF_1 by A—BBC	311	187	124	60.1%	426	626	
ABF_2 by A—BBC:							
Cancer F_2 mothers	223	140	83	62.8%	435	637	57.78% ± 7.27 or 7.95 × S.E.
Nonca. F_2 mothers	60	3	57	5.0%	480	715	
Total	283	143	140	50.5%	437	679	

COMPARISON OF OBSERVATIONS WITH GENETIC EXPECTATION

The development of spontaneous mammary cancer in mice has been demonstrated to result from the inciting influences of intrinsic and extrinsic causes. In this investigation only the animals that had received the agent transferred in the milk of females of the cancerous stock were used to determine the nature of the inherited susceptibility. For this purpose it has been assumed that the mice of the A stock were homozygous for the inherited susceptibility; mice of the low cancerous B stock were considered nonsusceptible.

There was no difference in the incidence of tumors recorded for the females of the A stock and the first generation hybrids nursed by females of the cancerous stock. Thus there was little doubt regarding the dominance of the inherited susceptibility.

The data secured in the F_2 generation were in accord with the genetic theory that a single factor might be involved. The incidence was 74.9 per cent for 391 animals, and the expected ratio for one factor would be 75 per cent. Because of this close agreement the other comparisons are based on the theory that the inherited susceptibility was a single dominant. For the present it has been assumed that the animals that died noncancerous were nonsusceptible.

Forty-two F_2 females were used to produce the F_3 by A hybrids. According to genetic theory, the segregation of a single factor in individuals of the F_2 generation should give:

One-fourth homozygous for the factor.

One-half heterozygous, and

One-fourth of the F_2 mice would be nonsusceptible.

Because of the small number of animals, it would be improbable that mice having these various genetic constitutions would be equally distributed. Thus the expected incidence of cancer for the mice of the F_3 by A generations was determined as follows:

[2] The difference between the A and F_1 by A mice was 2.74 per cent, or 1.83 × S.E.; the respective cancer ages were 285 and 341 days.

(a) All F_2 males were considered to be heterozygous for the cancer susceptibility factor.[3]

(b) Noncancerous F_2 mothers transmitted only the recessive alleles.

(c) Cancerous F_2 mothers were assumed to be heterozygous for the factor unless their progeny had an incidence of 91 per cent or higher (the average incidence in the mice of the F_1 generation and A stock was 94.5 per cent).

Based on this grouping, the expected incidence for the young born to cancerous mothers of the F_3 by A hybrids would be 76.2 per cent, whereas the recorded incidence was 70.9 per cent. The degree of significance for the difference, 5.3 per cent, was 1.9 × S.E. The expected incidence for F_3 mice with noncancerous F_2 by A mothers would be 50.0 per cent, and the observed incidence was 45.9 per cent (0.61 × S.E.). If cancerous mothers were considered to be homozygous when the incidence among their progeny was greater than 85 per cent, the expected incidence would be higher and the difference between this and the observed incidence would be mathematically significant.

All the ABF_1 mothers of the ABF_1 by A—EBC mice were theoretically heterozygous, and their progeny should have an incidence of 50 per cent if the inherited susceptibility was a single dominant. The observed incidence was 60.1 per cent and the difference between the ratios was 10.33 per cent ± 3.99, or 2.54 × S.E. By mothers, the incidences ranged among the progeny from 27.6 per cent to 83.3 per cent.

The genetic constitutions of the F_2 mothers used to produce ABF_2 by A—BBC mice may be considered approximately the same as the F_2 mothers that gave birth to the mice of the F_3 by A generation. One F_2 mother had progeny with an incidence greater than 80 per cent. Even assuming this female to be homozygous and the others heterozygous for the susceptibility factor, the expected incidence for the mice of this generation would have been only 52.9 per cent, or 9.87 per cent (2.11 × S.E.) below the observed incidence. The incidences varied, by cancerous mothers, from 45.0 to 84.6 per cent. Noncancerous F_2 mothers, when mated to males of the low cancerous stock, had progeny with an incidence of 5.0 per cent; expected incidence 0 per cent. The genetic cancerous susceptibilities of the ABF_1 by A and cancerous ABF_2 by A mothers were very similar as revealed by the respective incidences in the backcross animals (60.1 and 62.8 per cent).

Table III: Incidence of Mammary Cancer in Breeding Females of the A Stock and Their Hybrids Based on the Age at Which the Mothers Developed Cancer. All Mice Were Nursed by Females of the A Stock or Were Descended from Such Mice. Progeny of Noncancerous Mice Are Not Included. Totals Are Given in Table II

	Age of cancerous mothers								
	Under 300 days			301-400 days			401+ days		
Stock	No.	% Ca.	Ca. age	No.	% Ca.	Ca. age	No.	% Ca.	Ca. age
A by A	581	97.4%	280	332	93.1%	290	59	83.1%	318
ABF_1 by A and BAF_1 by A	110	93.6%	338	118	92.4%	341	6	66.7%	399
ABF_2 by A and BAF_2 by A	132	80.3%	399	162	75.3%	411	97	67.0%	436
ABF_3 by A and BAF_3 by A	82	79.3%	347	182	69.8%	372	244	68.9%	399
ABF_1 by A—BBC	78	55.1%	426	126	67.5%	432	107	55.1%	417
ABF_2 by A—BBC	13	84.6%	336	94	68.1%	434	116	56.0%	453

Thus, in every comparison, either the data did not differ significantly from the expected ratios or the observed incidences were higher than would be expected according to the theory that the inherited susceptibility for mammary cancer may be a single dominant factor.

Incidence by the Cancerous Age of the Mothers

Data have been published indicating that the age at which the mothers developed mammary cancer may influence the incidence of tumors in the progeny (12). Such data are given in Table III. The mothers were divided into three groups according to their age when tumors were observed: under 300 days, 301 to 400 days, and over 401 days. There was a tendency for the young born to these females to have a decreasing incidence of cancer with the increasing age of the mothers at the time of the development of their tumors. Also, the younger were the mothers when

[3] All matings between noncancerous F_2 females and F_2 males gave cancerous progeny, but no group gave results indicating that any male might be homozygous.

they became cancerous, the earlier did tumors appear in their progeny. The ABF_1 by A—BBC hybrids were the only exceptions. Several of the groups had differences in the incidences of cancer that were statistically significant.

Incidence of Mammary Cancer by Litters

From inspection of the records in these experiments it was apparent that there might be an increase in the incidence in the mice born in consecutive litters. To determine how the litters should be grouped for such a tabulation, mice of the F_2 and F_3 generations with cancerous mothers were combined to give as many animals as possible (899). The incidence for these groups was 74.9 and 70.9 per cent; the degree of significance was 1.36 × S.E. The following incidences were secured in the mice born by litters: first and second, 63.3 per cent, third and fourth, 78.6 per cent; and fourth and following litters, 77.5 per cent. Thus the mice were grouped according to those born in the first and second litters and the third and following litters. The number of mice in some generations was too small to make other divisions; also, mice developing tumors at an early age seldom had more than three or four litters. These results have been presented in Table IV.

Table IV: Incidence of Mammary Cancer in Mice Born in Successive Litters. Totals Are Given in Table II

	Born 1st and 2nd litters				Born 3rd and following litters			
			Average age, days				Average age, days	
Stock *	No.	% Ca.	Ca.	Nonca.	No.	% Ca.	Ca.	Nonca.
A by A	446	96.2%	286	431	526	94.1%	285	423
Total F_1 by A	143	88.1%	355	507	91	98.9%	321	212
Total F_2 by A	159	71.1%	400	573	232	77.6%	420	552
Total F_3 by A:								
Cancer F_2 mothers	173	56.1%	408	574	335	78.6%	370	539
Nonca. F_2 mothers	38	28.9%	414	546	71	54.9%	398	582
Total	211	51.2%	409	567	406	74.4%	373	552
ABF_1 by A—BBC	106	55.7%	410	658	205	62.4%	433	607
ABF_2 by A—BBC:								
Cancer F_2 mothers	66	60.6%	427	685	157	63.7%	439	615
Nonca. F_2 mothers	23	8.7%	477	725	37	2.7%	486	709
Total	89	47.2%	429	702	194	52.1%	439	652

* Total F_1 by A = ABF_1 by A and BAF_1 by A; see Table III.

Mice of the A stock had progeny with the same incidence and average cancer age regardless of the litter in which they were born.

Increases in the incidence of cancer were noticed in mice of all hybrid generations [4] when they were born in the third and following litters. However, the variations were great enough, with the number of mice studied, to be significant only in the F_1 and F_3 hybrids. In these groups, earlier average cancer ages were observed also for the animals with the higher incidences.

Summary of Experimental Data

Approximately 4,000 hybrid females descended from reciprocal matings between parents of the cancerous A and low cancerous B (C57 black) stocks were studied to determine the make-up of the inherited susceptibility for mammary carcinoma in mice. Few spontaneous tumors were observed in the mice nursed by females of the low cancerous stock. The hybrids descended from mothers nursed by females of the cancerous stock had incidences in accord with the genetic theory that the inherited susceptibility is transmitted as a single dominant. The incidence of mammary cancer was influenced by the age at which the mothers developed spontaneous tumors and by the litter in which the young were born.

DISCUSSION

Because mammary cancers were found to develop in a high percentage of the mice of some strains, whereas few animals of other stocks had tumors, it was asserted by the early workers that the inherited constitution of the host is one of the primary factors in the genesis of such growths. The role of the estrogenic hormones was recognized by studying the incidence in virgin, breeding, and ovariectomized females, by the transplantation of ovaries into castrated males, and by the injection of estrogens (for literature, see 13, 15, 19).

As soon as hybrid crosses were made between inbred strains of mice it was demonstrated that some

[4] Mice that had mothers from the B stock but were nursed by females of the A strain were classified by the litter born to the foster mother. No intrauterine influence was observed.

maternal or extrachromosomal influence also must be considered (25). This was found to be transferred in the milk of females of cancerous strains, and is probably a virus (5, 9, 11, 13, 27).

Following the discovery of the extrachromosomal influence, some doubt existed regarding the role of the intrinsic factor in the development of mammary cancer in mice. Murray and Little (23) concluded that the maternal influence is 6 to 10 times more important than any possible chromosomal factor and that the tendency to have mammary cancer is not mendelian in nature (24). As soon as experiments on foster nursing had been completed it was evident that inherited susceptibility was an important factor (7). Also, that tumors resulting in nonsusceptible animals may have a different etiology from those developing in susceptible mice (13). Andervont (1, 2) stated that there are different degrees of susceptibility to mammary cancer, which are influenced by factors introduced into hybrid crosses by males of resistant strains. Making use of foster nursing, Murray (22) concluded that the development of mammary cancer in mice of inbred strains and their hybrids "may be reasonably explained upon the basis of the strength of the milk stimulus which the mice receive and the resistance of the physiological systems produced by these matings to various concentrations of the milk stimulus." [5]

Murray failed to mention in this connection the action of the estrogenic hormones in the production of mammary cancer, although he was one of the first to point out their importance (20, 21).

As far as can be demonstrated from the data now available, hormonal stimulation, inherited susceptibility, and the milk agent are of approximately equal importance in the etiology of mammary cancer in mice of known genetic constitution under normal conditions (13). That is, any one of the three factors or influences may be completely determining in its effects. Susceptible mice without the milk agent will have approximately the same incidence as nonsusceptible animals, etc.

Recently Fekete and Little (14) have concluded that, in addition, an intrauterine influence may be involved in the production of mammary cancer. In their experiment fertilized ova from females of the C57 black stock were transferred to the uteri of females of the dilute brown cancerous stock. Fourteen C57 black females were born from transferred ova. They were nursed by the dilute brown females and received the active milk agent. One was kept as a virgin female and three were mated but had no progeny. All died noncancerous. The others were forced-bred, the "functional test" of Bagg (4), during part of the breeding period; 5, or 50 per cent, developed spontaneous mammary cancer. Ninety-three mice born to or descended from the transferred-ova C57 black females were found by Fekete and Little to have an incidence of 73 per cent. These animals would not have the uterine influence of the females of the cancerous stock.

Various incidences of cancer have been reported in mice of the C57 black stock. The incidence did not exceed 1 per cent in breeding females that nursed their young (17), or in forced breeders that did not have the active milk influence (18). When mice of this strain were fostered by females of cancerous strains, incidences from 9 per cent (22) to 13 per cent (26) have been reported in virgin females. The incidence described in this report was 18.4 per cent for fostered females that nursed their young. When foster nursing and forced breeding were employed, Bagg (3) reported an incidence of 50 per cent, and Andervont (2) found an incidence of 63 per cent in C57 black females. In analyzing such results it should be recalled that Gardner (16) found forced breeding to produce a higher incidence of mammary tumors in the same stock than did the injection of estrogenic hormones.

Any intrauterine influence should function in hybrid animals as well as in mice of inbred strains, but no evidence for such an effect could be found in hybrids of the cross between the cancerous A and low cancerous C57 black stocks (12). Murray (22) observed that those born of C57 black mothers and dilute brown fathers, but nursed by dilute brown females, gave an incidence of 65 per cent, whereas the hybrids born to and nursed by dilute brown females were reported by Murray and Little (23) to have an incidence of 40 per cent.[6]

In an attempt to determine how the inherited susceptibility for mammary cancer in mice may be inherited, reciprocal matings were made between mice of the cancerous A and low cancerous C57 black stocks. The A stock had been inbred for over 60 generations and over 45 successive generations of cancer have been observed. The mice of this strain were probably as homozygous for any character as they could possibly be made. In breeding females the incidence of spontaneous cancer was 95 per cent. None of the C57 black mice used as controls had tumors.

Mice of the first hybrid generation either nursed their mothers or were fostered by females of the

[5] To define the physiological system, Murray wrote: "If the genetic theory is accepted, each individual is a physiological system the make-up of which is primarily determined by its genetic constitution.

'Genetic constitution' is here interpreted as being the interaction of the total number of genes which the individual inherits."

[6] These data may not be comparable, since the two groups were observed at different periods and in different laboratories.

reciprocal strain. They were used as breeding females to insure an adequate estrogenic stimulation, as a previous experiment demonstrated that virgin females would develop few neoplasms (6).

Few of the hybrids nursed by females of the low cancerous stock had mammary carcinoma and thus were of little value in determining the nature of the inherited susceptibility. There was no apparent difference in the results of the animals born to mothers of the two strains, provided they did not receive the active milk influence.

There was no significant difference between the incidence of the first generation hybrids born to B stock mothers but nursed by females of the A stock, and those born to and nursed by mothers of the A strain. Likewise, the results in the F_2 and F_3 generations were similar. When the incidence for the entire F_1 population was tabulated it was found to be approximately the same as was observed for breeding females of the high cancerous stock. In the other hybrid generations the results either were in accord with the genetic theory for a single dominant factor, or more tumors were observed than would be expected.

When mice of the C57 black stock were nursed by their maternal parents mammary tumors either did not develop, or the incidence was very low. Because of their resistance, we have considered the mice of this strain to be nonsusceptible to the development of spontaneous mammary cancer. If mice of this strain were fostered by females of the cancerous A stock, the incidence increased to 18.4 per cent. If these mice were nonsusceptible, the active milk agent and the estrogenic stimulation were able to overcome the threshold of nonsusceptibility in nearly 20 per cent of the fostered C57 blacks and produce tumors.

In view of the fact that tumors resulted in some of the so-called nonsusceptible C57 black mice with the milk influence, it seemed probable that some of the nonsusceptible hybrids also had tumors. Because these hybrids were somatically cancerous, they were considered to be genetically cancerous; that is, they were tabulated as having inherited the susceptibility.

The mice of the F_2 generations with the active milk influence had an incidence of 74.9 per cent. How many of the animals were nonsusceptible and developed spontaneous tumors because of the action of the milk agent and the estrogens is problematical. As nearly 20 per cent of the C57 black mice with the milk influence had neoplasms, one might expect the same percentage of nonsusceptible F_2 by A hybrids to die cancerous. If this was the case, of the 391 F_2 animals, 293 would theoretically be susceptible if a single factor was involved and would be expected to develop mammary cancer, while 20 of the nonsusceptible mice might have succumbed to the disease. On this basis, we should expect 80.1 per cent to show the condition. This was 5.2 per cent higher than the observed incidence ($1.74 \times$ S.E.).

If the susceptibility for mammary cancer was due to two independent dominant factors, the incidence of the disease would be the same in mice of the susceptible stock and hybrids of the first hybrid generations with the active agent. According to the theory of independent assortment, there should be 9 genetically cancerous animals to 7 nonsusceptible mice in the F_2 population, or a cancerous percentage of 56.3 per cent. We might also assume that about 20 per cent of the nonsusceptible animals would give rise to cancer, which would increase the incidence for the group to approximately 65 per cent. Both these ratios would be below the observed incidence. If two factors were transmitted, 1 of 16 of the F_2 mice would theoretically have the same genetic constitution as the mice of the nonsusceptible stock. Six out of 16 of the group of F_2 nonsusceptible mice would have one of the susceptibility factors either in the homozygous or heterozygous condition. If about 20 per cent of the nonsusceptible C57 black mice with the milk agent gave rise to cancer, it should be justifiable to expect a higher incidence in the animals with one of the two susceptibility factors. Should the incidence approach 40 per cent, the incidence for the entire F_2 population would be about 73 per cent on the assumption that two dominant factors were involved. This ratio was approximately the same as the observed incidence.

The determination of the number of susceptibility factors was made more difficult by the fact that the incidence of mammary cancer in the breeding females of the inbred A stock, and in most of the hybrid generations, was influenced by the age at which tumors developed in the mothers and by the litter in which the progeny were born. In some groups the age of the mothers at the time they became cancerous seemed to be of more importance than the litter number. Generally the groups with the higher incidence had the lower average tumor age.

It is doubtful if all the mice used in the study had the same level of estrogenic stimulation, although they were all maintained as breeders. The animals with the largest number of litters presumably produced the most estrogens. Generally such mice had the oldest average tumor age. Thus it is improbable that the observed variations in the incidences of cancer could have resulted from differences in individual production of the hormones. The effect might be explained by assuming that the females with a more concentrated milk agent gave rise to cancer at earlier ages, and that the concentration of the agent was enhanced with the increasing age and reproductive activity of the females.

Although the data secured in the hybrids could best

be explained on the theory that the inherited susceptibility to spontaneous mammary cancer is transmitted as a single dominant, it is probable that multiple factors were involved. The inciting influences of the agent transferred in the milk of females of the cancerous stock, and the estrogenic hormones, functioned in overcoming the threshold of nonsusceptibility in a considerable proportion of these mice and resulted in the development of mammary carcinoma. This would imply that the etiological factors were not identical in the genesis of all growths of this type. The number of factors transmitted could not be estimated because it was impossible to determine how many of the cancerous mice were genetically noncancerous. If many factors were involved, the possibility existed that some were common to the mice of the two parental stocks.

CONCLUSIONS

As previously determined, the genesis of spontaneous mammary tumors in mice is dependent upon the inciting effects of the mammary tumor milk agent, the mammary tumor inherited susceptibility, and the mammary tumor estrogenic stimulation.

The determining effect of any one of these three factors or influences was about equal.

In this study the inherited susceptibility to spontaneous mammary cancer in mice was transmitted by males and females of the cancerous or susceptible stock.

The data obtained in the hybrid and backcross generations were in accord with the genetic theory that it might be a single dominant factor, but other explanations are possible.

The progeny of cancerous females of the second hybrid generation had a higher incidence of cancer than did the progeny of noncancerous mothers.

The incidence of mammary cancer in the progeny was influenced by the age at which the mothers developed tumors.

The litter in which the mice were born influenced the incidence of cancer.

The concentration of the milk agent may increase with increasing age of the mothers.

No intrauterine influence could be demonstrated in these studies.

A considerable percentage of the mice of a low cancerous or nonsusceptible strain that received the active milk agent developed mammary carcinoma.

This observation probably indicates that some of the cancerous animals of the hybrid generations were genetically noncancerous or nonsusceptible.

Because some hybrid mice that were somatically cancerous were not genetically cancerous, it was impossible to determine the number of factors transmitted but it is probable that multiple factors are involved.

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Chromophobe Adenoma-Like Lesions of the Rat Hypophysis

Frequency of the Spontaneous Lesions and Characteristics of Growth of Homologous Intraocular Transplants*

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(Received for publication October 20, 1943)

In a study of spontaneous diseases that occur in albino rats with advance in age, chromophobe adenoma-like lesions were found in the hypophyses of many old rats of the Yale strain. The frequency of this lesion in a limited series of animals, and the preliminary results of intraocular transplantation of one of the lesions, have been reported (8). Periods of 7 to 12 months elapsed before growth of these transplants became evident, and it was therefore thought likely that advanced age of the host was necessary for growth of this type of tissue following transplantation. This is contrary to the usual experience that transplanted tumors grow best in young animals, and would suggest that this tissue had little if any autonomy of growth and therefore was not neoplastic.

Experiments have been continued to determine so far as possible by the behavior of transplants whether the spontaneous growths represent hyperplasia of chromophobe cells in response to endogenous stimuli associated with advancing age, or true neoplasia in the generally accepted sense. The experiments were designed to study the character of growth of transplants under varying conditions of age, sex, and strain of the hosts. For comparison, transplants were made of normal hypophyseal tissue. The study of frequency of the spontaneous lesions has been continued to include a larger number of rats of the Yale strain. In addition, the pituitaries of rats of the Sherman strain and of a stock derived from the Wistar albino strain have been examined.

MATERIALS AND METHODS

Three hundred sixty-two albino rats of the Yale strain,[1] ranging from 1 month to about 3 years of age, are included in the study of frequency of spontaneous chromophobe adenoma-like lesions. The pituitaries of all animals were examined in the gross, and the great majority were sectioned and stained with hematoxylin and eosin, and with Heidenhain's modification of Mallory's aniline blue technic (6). The pituitaries of 170 rats of the Sherman strain,[2] and of 79 rats of a stock derived from the Wistar albino strain[3] were also examined. A smaller proportion of these were sectioned, and frequency of the lesions is based chiefly upon the gross appearance of the pituitaries. Enlarged pituitaries were rarely seen in rats of these strains.

Intraocular homologous transplantation was carried out in the following manner. The rat was anesthetized with chloroform, the upper part of the cornea was incised with a safety razor blade, and the opening widened with small scissors. Chloroform was preferable to ether because it produced greater dilatation of the pupil and consequently there was less chance of hernia of the iris during manipulations. Pieces of tissue less than 1 mm. in greatest diameter were introduced into the anterior chamber and pressed into the inferior region of the angle of the iris. In a few instances tissue was transplanted into the subcutaneous tissue and into the testis, but none of these transplants was observed to grow. Tissues from 4 spontaneous lesions were transplanted. The transplants from 2 of the 4 lesions, 1 each from the Sherman and Wistar de-

* This investigation was carried out with the aid of a grant from the Josiah Macy, Jr., Foundation. Presented in part at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, held at St. Louis, Missouri, April 3, 1942.

[1] Rats of the Yale strain, which is derived from the Osborne-Mendel strain, were obtained from the stock colony of the Department of Animal Nutrition at Cornell University. Some had been used in experiments dealing with the relation of diet to the life span, but could be considered normal for the purposes of this survey. We are indebted to Dr. C. M. McCay for the rats of this strain.

[2] Most of the rats of the Sherman strain, which is a derivative of the Osborne-Mendel strain, were obtained from the Rockland Farms. Others were raised in this Department. Some had been used in experiments where the procedures would not have been likely to influence the frequency of hypophyseal lesions.

[3] These rats were from a stock that was formerly maintained in the Department of Biochemistry of Cornell University Medical College. The animals from which this stock originated were of the Wistar albino strain. We are indebted to Dr. N. F. Blau for rats of this stock.

rived strains, respectively, did not grow. Transplants from lesions in 2 rats of the Yale strain grew in a number of the recipients, and were carried into second and third serial generations by intraocular transfer. These form the basis for analysis of the behavior of the hypophyseal lesions after transplantation. Intraocular transplants of normal hypophyseal tissue were made in the same manner to serve as controls. The details of individual experiments are described with the results.

RESULTS

Frequency and Morphology

Yale strain.—The frequency of chromophobe adenoma-like lesions of the hypophysis in rats of the Yale strain in relation to age and sex is shown in Table I.

Table I: Frequency of Chromophobe Adenoma-Like Lesions in Rats of the Yale Strain in Relation to Age and Sex

	Males			Females		
Age, days	Number of rats examined	Rats with lesion: number	Rats with lesion: per cent	Number of rats examined	Rats with lesion: number	Rats with lesion: per cent
0-200	67	0	0	12	0	0
201-400	37	2	5.4	25	0	0
401-600	65	34	52.3	47	6	12.8
601-800+	56	34	60.7	53	16	30.2

Adenoma-like lesions occurred in 92 of 362 hypophyses examined. Two lesions only were found in rats less than 400 days old. These were microscopic in size and occurred in males aged 237 and 241 days respectively. The frequency increased with advancing age to about 60 per cent in males and 30 per cent in females 600 and more days old.

The lesions included in Table I varied from small nodular areas of chromophobe hyperplasia seen only in microscopic section, to large adenomatous growths of chromophobe cells, the largest weighing 367 mgm. Multicentric origin was common, and the microscopic appearance in most instances resembled that already described (8). The nodules were not encapsulated and did not appear invasive. They were characteristically composed of cords and irregular groups of cells with chromophobic vacuolated cytoplasm and with vesicular nuclei, often of large size, in which the chromatin was usually in the form of coarse granules. Many nuclei contained large eosinophilic nucleoli. A few of the growths were cavernous in structure, whereas most were solid. The former observation that the cytoplasmic vacuoles contained fat was confirmed. Several growths were composed of small cells with relatively scant chromophobic cytoplasm in which no vacuoles were seen. Although differing in appearance from the others, these were considered to be chromophobe adenoma-like lesions on the basis of staining properties, and are included in Table I. It was not possible to trace the origin of any of the lesions observed; but since many of the smaller nodules were embedded deeply in the anterior lobe of the pituitary, it seemed probable that they developed from cells of this lobe. No chromophilic growth was seen in the entire series. The two lesions from which transplants were made were typical of the majority, being composed of rather large cells with chromophobic vacuolated cytoplasm.

Sherman strain.—The age distribution in rats of the Sherman strain was about the same as that in the Yale strain. There were 93 rats, 83 males and 10 females, aged from 1 to 3 years, and 77 rats less than 1 year old. Three chromophobe adenoma-like lesions occurred in this series, all in male rats between 2 and 3 years old. The largest lesion measured 6 mm. in diameter and was composed of chromophobic vacuolated cells with large nuclei containing coarse granules of chromatin. Many large and small vascular spaces were within the lesion. A slightly smaller growth, from which transplants were made, was composed of cells with less cytoplasm and without vacuoles. The nuclei varied somewhat in size and staining properties. Vascular spaces were numerous and usually small. One large organizing thrombus was present. Small fragments of this lesion were transplanted into the eyes of 2 rats of this strain, and into the eyes of 3 males and 1 female of the stock derived from the Wistar strain. All recipients were about 18 months old. No growth was seen at any time, and the rats were discarded after 5 months of observation. The third adenoma-like lesion consisted of a nodule 2 mm. in diameter located in one side of the hypophysis. It was composed of small cells without vacuoles and contained abundant small vascular spaces. There was an infiltration by a few phagocytes laden with brown pigment.

Stock derived from the Wistar strain.—Of the 79 rats of this stock, 13 were over 1 year of age when examined. There were 9 males and 4 females. A large tumor of the pituitary was found in a male rat 17 months old. The pituitary bearing the lesion weighed 191 mgm. The tumor was composed of chromophobic cells without vacuoles, and contained small areas of fresh hemorrhage. Part of the tissue was transplanted to the eyes of 7 male and 5 female rats of the same stock, aged 2 months. In no instance was any growth observed, and the recipients were discarded after periods of 8 to 13 months.

Transplantation

Chromophobe adenoma-like lesions.—The results of transplanting one spontaneous adenoma-like lesion from a rat of the Yale strain are shown graphically in Chart I. The first serial transplants of this growth have been described (8). The original lesion was found in a male rat 17 months old. After prolonged

latent periods 3 of the 9 intraocular transplants began to grow slowly. Two of these were used for second serial transfers. In one instance the tissue was transplanted to the eyes of 6 male rats of the Wistar stock 5 months old, with resultant growth in 2 of the rats. In the other case, 21 months after the first transfer, tissue was placed in the eyes of 4 male rats of the Sherman strain 2 months old, and of 4 albino rats of unknown strain, about 18 months old. Growth of the transplants occurred in 3 of the 4 old rats and in 2 of the 4 young rats. After periods of 11 and 14 months the transplants in the younger animals had each reached a diameter of 3 mm. Third serial transplants were made in each case into the eyes of young male

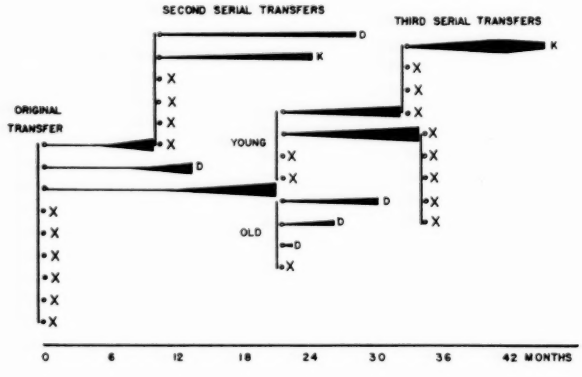


CHART I.—Outline of transplantation of chromophobe adenoma-like lesion from rat M264. Relative size of the transplants is indicated by the thickness of the lines. Time in months is shown along the baseline. The letter "x" indicates that transfer was considered negative at time the host was discarded. The letters "K" and "D" indicate whether the rats bearing the transplants were killed or died of natural causes.

rats of the Sherman strain. Growth resulted in 1 of 4 rats receiving tissue from one transplant, but in none of 5 rats receiving tissue from the other. Tissue of this transplant had the typical appearance of the original adenoma-like lesion, but there was in addition an infiltration of lymphocytes. The rat bearing this transplant was killed because of labyrinthitis, and the right middle ear was found to contain pus. It seemed probable that contamination of the tissue had interfered with growth of the transplants, and the 5 recipients are therefore not included in the analysis of the transplantation experiments. The rat bearing the only successful third serial transplant was observed for 13 months. The transplant reached a diameter of 3.5 mm. after 8 months, but during the 12th and 13th months it decreased in size and the color changed from yellowish pink to white. Microscopically, there was degeneration of the transplanted tissue, but the structure of the original lesion could be recognized.

In all transplants where growth was observed the tissue retained the microscopic structure of the original lesion and became vascularized as evidenced in the gross by a pink color and in section by capillaries filled with blood. The transplants usually became surrounded by a thin capsule of fibrous tissue. In this series it was shown that chromophobe adenoma-like tissue would grow after homologous intraocular transplantation; that growth of transplants was extremely slow and might follow long periods of latency; that growth of adenoma-like tissue would occur in second and third serial intraocular transfers; that growth would take place in the eyes of rats of strains other than that in which the original lesion developed; and

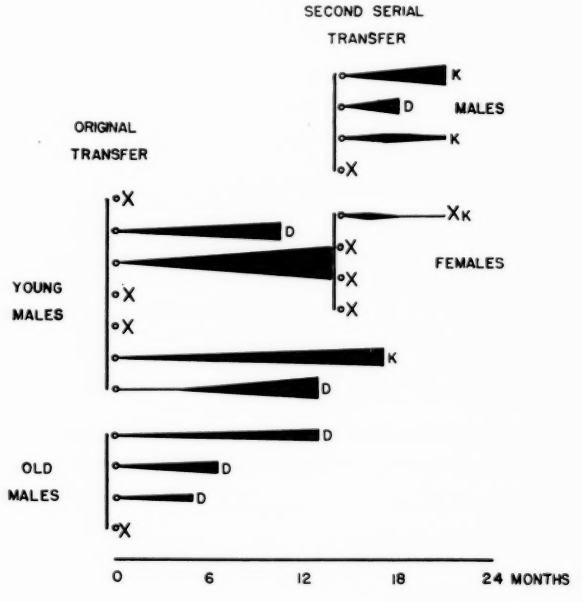


CHART II.—Outline of transplantation of chromophobe adenoma-like lesion from rat W-D 142. Symbols are the same as for Chart I.

that intraocular transplants would grow as well in young as in old rats of the male sex.

The second chromophobe adenoma-like lesion to be successfully transplanted occurred in a male rat of the Yale strain 17½ months old. The lesion was estimated to weigh about 70 mgm. Tissue was placed in the eyes of 4 male rats aged 18 months and of 7 male rats aged 7 weeks, all of the Yale strain. The behavior of the transplants is shown in Chart II. Growth occurred in 4 of the 7 young rats and in 3 of the 4 old rats. The transplants increased in size more rapidly in the young than in the old animals. In general the period of latency was not so conspicuous as in the case of the first lesion transplanted (Chart I). The transplants did not differ appreciably in microscopic appearance from the original lesion, which is illustrated in Fig. 1. One transplant in a young rat measured 5 mm. in diameter 14 months after transfer, and weighed 25 mgm. This transplant in the eye is shown in Fig. 2. Tissue from it was put into the eyes of 4 young male and 4 young female rats of the Yale

strain. Transplants grew well in 3 of the 4 males, but in one instance there was some regression in size after 4 months of progressive growth. One of these second serial transplants in a male rat, killed 8 months after transfer, is shown in Fig. 3. In but 1 of the females was there growth of the transplant. It reached a diameter of slightly more than 1 mm. in 2 months, but after 4 months had regressed completely. When the recipient was killed 8 months after transfer no tissue could be found in the eye.

From this series it appeared that spontaneous adenoma-like lesions of relatively small size were capable of growth after homologous intraocular transplantation; that the growth of transplants was somewhat more rapid in young than in old animals; and that the male rat was a more suitable host than the female.

The two series of experiments were analyzed together to determine the effect of age and strain of the recipient upon the fate of the transplants. Excluding one group of 5 rats, referred to above, which received tissue that was probably infected, there were 21 successful growths in a total of 42 male rats receiving intraocular transplants.

Transplants grew in 15 of 34 rats of less than 1 year of age at the time of transplantation, whereas growth occurred in 6 of 8 rats that were over 1 year of age. Many of the transplants into young animals grew more rapidly and attained greater size than did those in old animals. It is therefore doubtful if age of the host exerted an appreciable influence upon the fate of transplants. The difference in proportion of successful transplants between young and old animals may perhaps be explained by the fact that the eyes of the young rats were smaller and thus presented a greater mechanical difficulty during inoculation.

The results of transplantation were analyzed in relation to the strain of the recipients. Growth of transplants occurred in 13 of 24 male rats of the Yale strain and in 8 of 18 albino rats of other strains. Thus the strain of the host was not an important factor in the survival and growth of transplants.

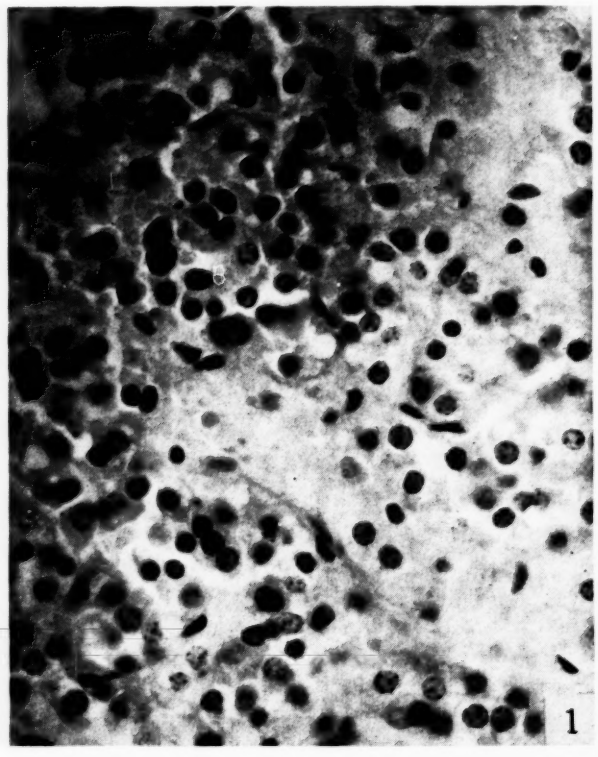
The relation of growth of transplants to the development of adenoma-like lesions in the pituitaries of the hosts was determined in the two series of experiments. Because spontaneous lesions were rare in pituitaries of rats of other than the Yale strain, these are excluded from the analysis. It was also necessary to exclude animals less than 1 year of age when examined, because of the observed low frequency of spontaneous lesions in young rats. Many of the recipients in which the transplants did not grow had been discarded after observation for 6 or 8 months, and before they were 1 year old. No animal excluded from the analysis bore a spontaneous adenoma-like lesion. There remained 14 old male rats for this analysis. Adenoma-like lesions were present in the hypophyses of 3 of 4 animals in which the transplants failed to grow, and in the hypophyses of 6 to 10 rats in which growth of transplants took place. It would appear from this small series that growth of transplants is no more probable in rats that develop spontaneous hypophyseal lesions than in those that do not. Moreover, it is noteworthy that the frequency of adenoma-like lesions of the hypophysis was not influenced by the inoculation of adenomatous tissue. The frequency of hypophyseal lesions in rats receiving transplants was of the same magnitude as that for the entire survey.

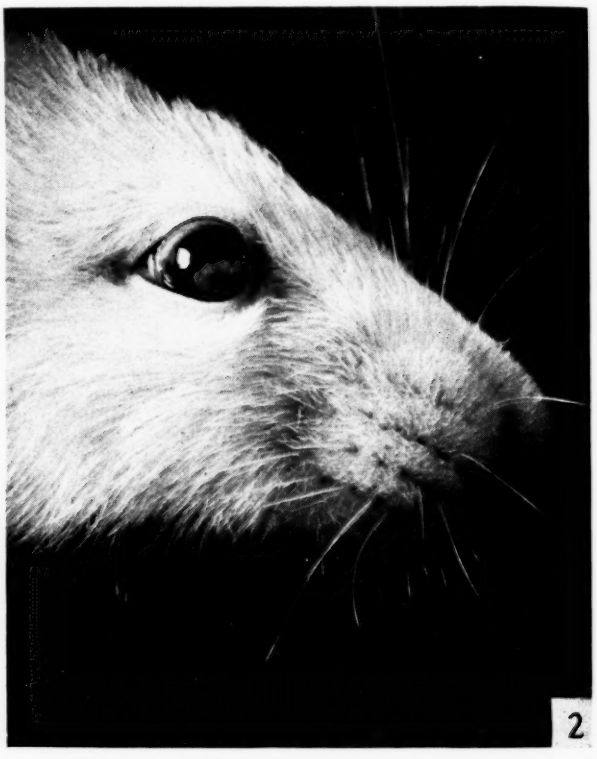
Normal hypophyseal tissue.—To serve as a control, anterior pituitary tissue from a male rat of the Sherman strain, aged 3 months, was transplanted into the eyes of 6 male rats aged 6 to 8 months, and of 5 male rats aged 3½ months. All animals were of the Sherman strain. The transplants were observed over a period of 7 months, and tissue persisted in the eyes of 9 rats throughout the period of observation. During the first 4 months the transplants appeared in the anterior chambers as small rounded pink masses between 0.5 and 1.0 mm. in diameter; subsequently, most of the transplants shrank and became white. One reached a diameter of 1.5 mm. after 1 month, but later decreased in size and at the end of the period of observation appeared as a tiny white speck. After 7 months only two transplants appeared viable. Each was about 0.5 mm. in diameter. One of these was sectioned and was found to be a rounded, encapsulated, and vascularized mass of cells bearing a closer resemblance to normal hypophyseal tissue than to the adenomatous growths. The cells were small, their nuclei of uniform size, and eosinophilic granules were present in the cytoplasm of many cells, usually those near capillaries. The transplant is shown in Fig. 4.

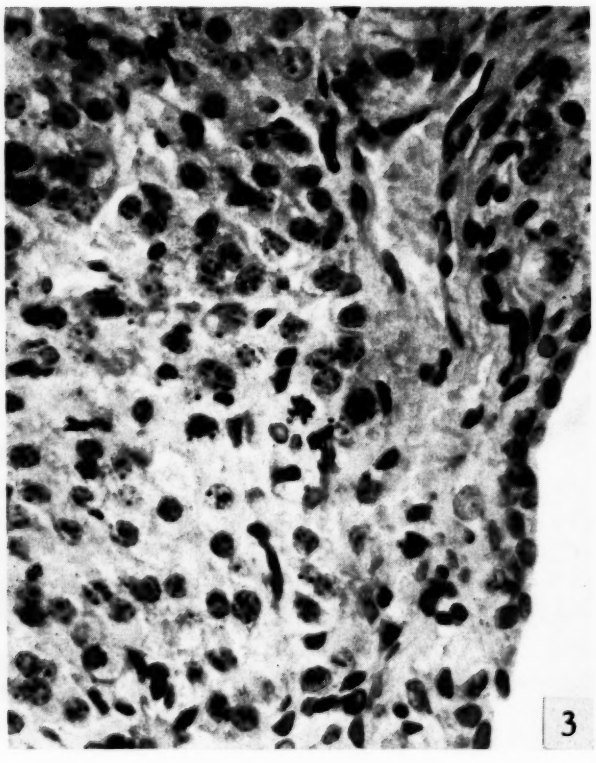
It is concluded from these observations that normal hypophyseal tissue may persist for a considerable period after homologous intraocular transplantation, but may not grow in a manner comparable to adenomatous tissue.

DISCUSSION

In these investigations an attempt has been made to determine whether the nodular chromophobic lesions of the rat's hypophysis are essentially hyperplastic or neoplastic in character. The behavior of the first serial transplants of one lesion, described in the former report (8), indicated that the tissue has little if any autonomy of growth, and was therefore merely hyperplastic. Studies of the morphology and frequency of the lesions, and of the behavior of subsequent transplants, reported here, have indicated that the lesions may be more closely identified with neoplasia than with focal hyperplasia. Nevertheless, the lesions do not completely satisfy the accepted criteria for neo-







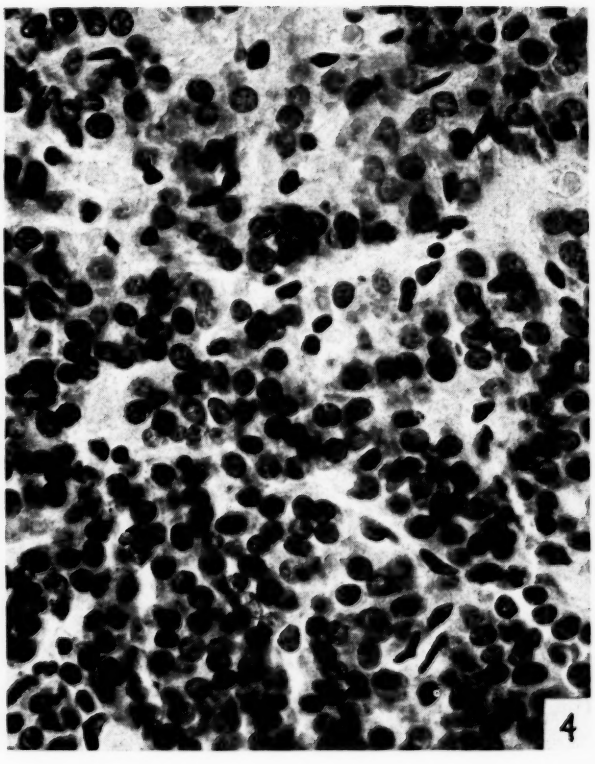


FIG. 1.—Original adenoma-like lesion from rat W-D 142. × 460.
FIG. 2.—First serial transplant in eye of rat (W-D 142-3), taken about 12 months after transfer.
FIG. 3.—Second serial intraocular transplant from rat W-D 142 (W-D 142-3-1), removed 8 months after transfer. × 460.
FIG. 4.—Intraocular transplant of normal hypophyseal tissue 7 months after transfer. × 460.

plasms. The term "adenoma-like lesion" has therefore been used again to describe the entire group, although it is reasonably sure that the largest lesions are adenomas.

On the basis of structure it has been difficult to classify many of the smaller lesions as hyperplastic or neoplastic. Wolfe, Bryan, and Wright (11) have described two main types of adenomatous reaction in the anterior pituitaries of old rats. Lesions of one type, considered by these authors to be adenomatous foci, ranged in size from 60 μ to 0.6 mm. and were composed of small groups or nests of enlarged chromophobic or occasionally eosinophilic cells. There was no encapsulation and no compression of surrounding tissue. Others, designated as true adenomata, ranged from 0.4 to 4.0 mm. in size and were composed almost exclusively of large chromophobic cells. These were not encapsulated, but were well demarcated from the normal tissue, which was compressed to a variable degree depending upon the size of the lesions. Numerous vascular spaces and areas of hemorrhage were described and the authors have classified these growths as hemorrhagic adenomata (11). The majority of lesions of our series, including those that were transplanted successfully, were generally similar to the hemorrhagic adenomata. Small vascular spaces were common, but areas of recent or old hemorrhage were noted only exceptionally. There was in all cases some distortion of the adjacent normal pituitary tissue. Cells of the smallest lesions of our series were not appreciably different from those of the largest. The small nodules would therefore seem to be young stages of the growth, and it is probable that the morphologic character of the lesion is determined early in its development. In favor of neoplasia is the observation that there was considerable variation in size and staining properties of the nuclei. Invasion of adjacent structures was not observed in any case, but it is significant that the largest lesions produced some erosion of underlying bone. In no instance was there a connective tissue capsule or other evidence of cellular reaction to the presence of the lesion, whereas the formation of a capsule is an almost constant feature of adenomata. The absence of a capsule may perhaps be taken rather as evidence of a low order of neoplasia than as evidence that the lesions are not neoplastic.

In our series, adenomatous changes of the pituitary were observed in but 2 rats less than 1 year of age. Wolfe and his associates (9, 10, 11, 14) have described adenomatous changes only in old rats. It may be inferred either that stimuli resulting in adenoma-like growth must act over a considerable period of time, or that changes occur in older animals favoring their development. The observed distribution in relation to sex would suggest that gonadal hormones may influence the occurrence of the lesions. Experiments of Cramer and Horning (1); McEuen, Selye, and Collip (7); of Wolfe and Wright (13); and of Zondek (15), where chromophobic lesions of the pituitary have developed after long-continued administration of estrogens, support this view. Furthermore, Wolfe (9) has found that adenomatous changes were rare in the anterior pituitaries of old male rats injected daily with testosterone propionate as compared with controls of the same age.

Adenomatous lesions were present in the pituitaries of 56 per cent of old male and of 22 per cent of old female rats of the Yale strain, whereas only 3 of 83 males of the Sherman strain, comparable in age, bore such lesions. Wolfe and his associates (9, 10, 11, 13) have found adenomatous changes in the pituitaries of 14 per cent of males and of 28 per cent of females of the Vanderbilt strain 17 and more months of age. The differences in frequency can be considered significant in that large numbers of animals of each strain were examined. Since the three strains of rats are all derived from the Osborne-Mendel strain, the frequency of adenomatous lesions would appear to have been influenced either by environmental conditions or, less likely, by selective breeding. In neither colony from which our rats were obtained has there been selection for purposes other than to maintain a vigorous stock. The Vanderbilt strain has been maintained by inbreeding (11). The number of animals of the Wistar derived stock that we examined is too small to warrant conclusions. A single adenomatous lesion was found in 1 of 9 old males of this stock. Wolfe, Bryan, and Wright (11) found pituitary lesions in 26 of 38 old females of the Wistar strain, or 68 per cent. In 11.4 per cent of old female rats of the Albany strain, Wolfe and Wright (14) noted adenomatous changes. It is thus apparent that while the lesions are widely distributed in common strains of rats their frequency in relation to strain and sex varies considerably.

The development of pituitary adenoma-like lesions does not appear to be associated with changes in other endocrine organs. No consistent change has been found in animals of our series in which the lesions have developed, although enlarged prostates and seminal vesicles were occasionally present. The Albany strain has been shown by Wolfe and Wright (14) to be characterized by structural and functional abnormalities of the ovaries, and by a high frequency of mammary tumors. Nevertheless, the frequency of adenomatous lesions of the pituitary was found by these authors to be lower than in strains with a normal reproductive function. Harned and Cole (3) have shown that rats of the Yale strain exhibit a lower glucose tolerance, more rapid growth, larger size, and a greater incidence of sterility than do those of the Wistar strain. These authors suggest that hyperfunction of the pituitary may be a common denominator

for these and some other differences between the two strains. However, Wolfe, Bryan, and Wright (11) have found pituitary lesions in 68 per cent of females of the Wistar strain, whereas we have found pituitary lesions in only 22 per cent of Yale strain females of the same age. Comparable data for males of the Wistar strain are not available. Although differences in endocrine constitution may exist among the various strains studied, these cannot be correlated with the frequency of pituitary adenomata.

The behavior of transplants has given a better indication of the nature of adenoma-like lesions than could be obtained from studies of frequency and morphology of the spontaneous lesions. From the behavior of three transplants described in the first paper (8) it appeared possible that the tissue would grow only in animals of advanced age, where factors perhaps favored the development of spontaneous lesions. It was therefore necessary to determine to what degree the tissue was dependent for growth upon age, sex, and strain of the host. It was important also to determine whether or not growth of transplants took place more readily in animals that developed spontaneous adenoma-like lesions.

It is of some interest that growth of adenoma-like tissue occurred only when this was inoculated into the anterior chamber of the eye. Subcutaneous and intratesticular transplants were introduced but did not appear to grow. The brain and the anterior chamber of the eye represent somewhat specialized sites for the growth of alien tissues in that they appear to be protected by the hematoencephalic barrier (4). Tissues placed in these regions are perhaps less subject to various defenses of the body, and for this reason growth of intraocular transplants may be less conclusive evidence of autonomy than growth after subcutaneous transplantation. However, transplanted tissues that grew had become vascularized, and so were in intimate contact with the blood of the hosts during their growth. Furthermore, the fact that intraocular transplants of normal hypophyseal tissue did not behave like the transplants of adenoma-like tissue can only be interpreted as evidence that the adenoma-like tissue possessed a capacity for growth greater than and independent of that of normal tissues. It is also of significance that normal hypophyseal tissue transplanted to the anterior chamber of the eye underwent much the same changes in appearance as were found by Wolfe, Kirtz, and Loeb (12) in anterior pituitaries of inbred mice transplanted into the subcutaneous tissue of their brothers and sisters. It is thus reasonable to assume that, within certain limits, the site of inoculation is of secondary importance in the behavior of transplants.

In our experiments transplants of adenoma-like tissue behaved in more respects like neoplastic than normal tissue. The growth rate, while characteristically slow, appeared to be independent of the age and strain of the recipients. Greene (2) has described a neoplasm of the rabbit kidney which, while bearing a close morphological resemblance to rapidly growing embryonal renal tumors of man, has been found to grow very slowly after homologous intraocular transplantation. A slow rate of growth after transplantation may thus be characteristic of some neoplasms. Only in respect to sex did transplants of adenoma-like tissue appear to be dependent upon constitutional factors of the host. Tissue did not grow so well in female as in male rats, although the number of females used as recipients was too small to be conclusive. The experiments were terminated before this difference had been adequately tested. For the same reason it is not possible to state that pituitary adenoma-like tissue can be carried indefinitely by serial intraocular transfer. It is conceivable that a hyperplastic tissue may upon transplantation carry with it the factor from the host inciting its original growth. However, such a factor would of necessity become diluted in subsequent passages if it were not a component or product of the tissue. The energy or rate of growth of subsequent serial transplants would thus be expected to diminish. That this was not the case in our experiments is shown by the fact that there was no diminution in the growth rate of second and third serial transplants. At the time the experiments were discontinued it could not be said that the limit of transplantability of adenoma-like tissue was being approached.

The growth of intraocular transplants appeared to be unrelated to the development of adenoma-like lesions in the pituitaries of the hosts. The tissue grew as well in the strains characterized by a high frequency of pituitary adenoma-like lesions as in strains where the spontaneous lesions were relatively infrequent. It was thus probable that the transplanted tissue did not derive a stimulus for growth from factors of the host that gave rise to the spontaneous lesions, and this may be taken as further evidence of autonomy. Moreover, the presence of growing transplants of adenoma-like tissue did not influence the frequency of pituitary lesions in the hosts. It is therefore unlikely that the tissue carried with it a separable agent that might stimulate growth of chromophobic cells.

From the studies of their behavior after transplantation, it is concluded that the larger growths of chromophobic cells are neoplastic in character. The frequency and morphology of the lesions are compatible with this view. It is doubtful, however, if the smaller lesions are neoplasms. These may represent hyperplasia in response to stimuli from within the endocrine system, since it is well known that disturbances in hormonal balance may induce proliferative changes in tissues upon which various hormones

may act. Hyperplasia of endocrine origin may lead directly to true autonomy of growth, or may provide a suitable substrate in which other factors may then induce neoplasia (5). When chromophobe hyperplasia as such ends and neoplasia begins was not determined in these experiments. It is probable that a study of transplants from very small or early chromophobic lesions would be of value in this regard.

SUMMARY

Chromophobe adenoma-like lesions of the hypophysis were found in 92 of 362 albino rats of the Yale strain. Two lesions only were observed in rats less than 1 year of age. The lesions increased in frequency with advancing age, and were present in 60 per cent of male rats and in 30 per cent of female rats 600 and more days old. The lesions varied in size from small nodules seen only in microscopic section to large masses, the largest weighing 367 mgm. The majority of lesions were composed of cells with vacuolated chromophobic cytoplasm and large oval nuclei in which the chromatin was usually in the form of coarse granules. Chromophobe adenoma-like lesions were found in the hypophyses of 3 of 83 male rats of the Sherman strain, aged between 1 and 3 years, and of 1 of 9 male rats of a stock derived from the Wistar strain, of comparable age.

Homologous intraocular transplants were made with adenoma-like tissue from two spontaneous lesions in male rats of the Yale strain, and these were carried in males into second and third serial intraocular generations respectively. Including all generations, growth of transplants occurred in 21 of 42 recipients. The transplants grew at an extremely slow rate, requiring from 11 to 19 months to reach a size suitable for serial transfer, and there was frequently a latent period of several months before growth was manifest. All transplants that grew became vascularized. The growth rate was not accelerated in subsequent transfers and the morphology did not change. Although the spontaneous lesions developed only at an advanced age, intraocular transplants grew as well in young as in old male rats. However, there was but slight growth in 1 of 4 female recipients. The strain of the recipient was found not to be a factor in either the percentage of successful transplants or their growth rate. The presence of spontaneous adenoma-like lesions in the pituitaries of the hosts did not influence the fate of transplants of adenoma-like tissue.

Normal hypophyseal tissue persisted for a considerable period after intraocular transplantation, but did not grow in a manner comparable to adenoma-like tissue.

Morphological studies have indicated that chromophobe adenoma-like lesions of the rat's hypophysis are more probably neoplastic than hyperplastic in nature. It is concluded on the basis of the behavior of transplants that the larger adenoma-like lesions are true neoplasms in that the growth rate of transplants, while characteristically slow, is independent of the growth rate of tissues of the recipients, and in that the ability of transplants to grow is independent of constitutional factors associated with age and strain of the recipient.

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Carcinoma of the Adrenal Cortex in a Rabbit

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(Received for publication September 15, 1943)

During the course of routine pathological examinations of 9 rabbits injected with relatively large amounts of the dye T-1824 (Evans blue), which is used for the determination of total plasma protein (Gregersen, Gibson, and Stead) and which has also been employed in therapeutic attempts in experimental cancer research (Brunschwig, Schmitz, and Jennings; Duran-Reynals), a bilateral tumor of the adrenals was found in one rabbit. True adrenal blastomas in rabbits are, according to Löwenthal, extreme rarities, as he has seen only a few lipoid-containing cortical adenomas or accessory cortical nodules. Boycott and Pembrey reported a doubtful, round cell sarcoma which allegedly originated from the adrenal from which, however, it was separated by a capsule. The bilateral adrenal neoplasms in the case to be presented are placed on record, as they are apparently the first true malignant adrenal blastomas of cortical derivation which have been observed in the rabbit, and as the occurrence of adrenal tumors in gonadectomized male and female animals has focused special attention on these neoplasms (Hueper and Martin).

The tumors were found in a male rabbit, weighing 2.4 kg., which had been injected intravenously with 3 cc./kg. of an 0.5 per cent Evans blue solution and which was killed approximately 6 months later. The postmortem examination showed that the upper incisor teeth were bluish in color; the testes were blue; the left adrenal was enlarged to about twice normal size and of a peculiar brown color, while the right adrenal was blue and attached to the pancreas and duodenum. The other organs were grossly normal with the exception of the testes, which were small.

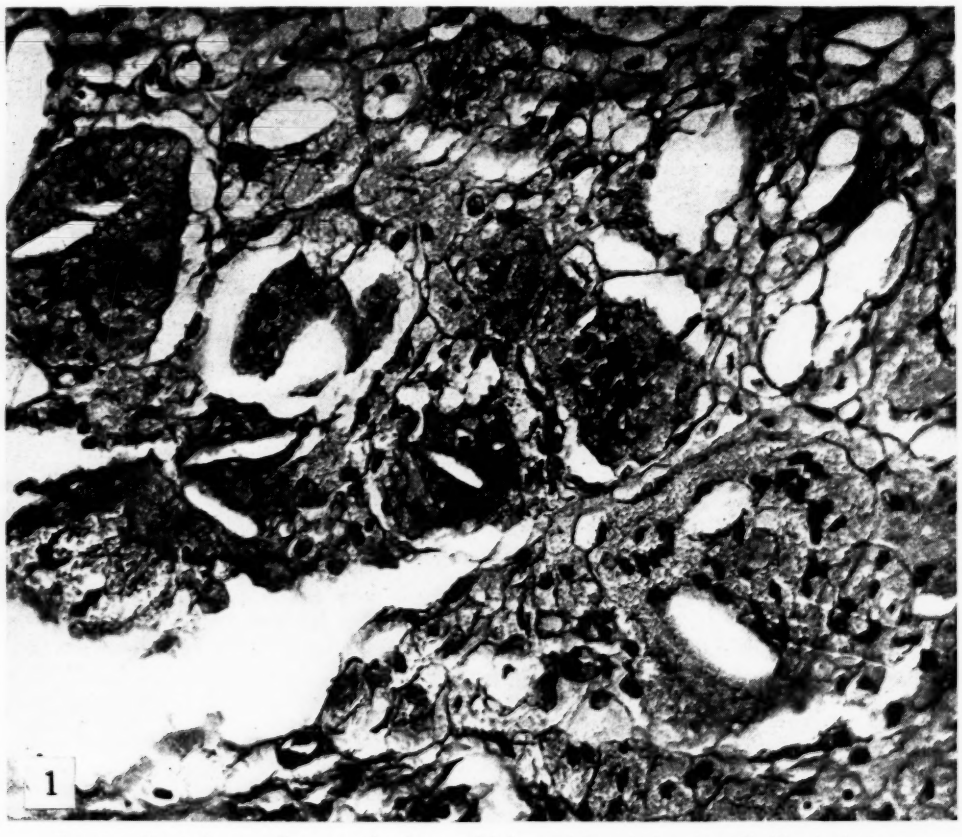

FIG. 1.—Huge foam cells, some having multiple nuclei and showing slit-like inclusions.

The histological examination of the adrenals revealed that the tissue of the left adrenal was placed like a triangular cap upon a small bean-sized mass consisting of huge foam cells containing in places numerous small, oval nuclei (Fig. 1). There were

scattered throughout the intact tumor tissue small slit-like crevices such as result from crystalline cholesterol deposits. The tumor was not demarcated by a capsule from the normal adrenal but merged directly with it. The rather thin fibrous capsule surrounding the other parts of the tumor was in places penetrated by small groups of the large foam cells which invaded the surrounding fat and reticular endothelial fibrous cushions. The liver and duodenum were hyperemic. The spleen contained areas with a thick hyaline network involving mainly the lymph follicles and the surrounding tissue, which contained large multinucleated giant cells often possessing a large vacuole. Similar hyaline matter was present in the renal glomeruli, while large albuminous casts were found in the distended cortical tubules.

FIG. 2.—Foam cell neoplastic infiltration into the periadrenal tissue.

connective tissue (Fig. 2). The central portions were occupied by large necrotic granular masses. The right adrenal was completely replaced by an identical tumor tissue which advanced into the adherent subserosa of the duodenum.

The testes were completely atrophic. The seminiferous tubules were often represented by very narrow lumina surrounded by thick hyaline rings embedded in a thickened fibrous tissue (Fig. 3). In other parts collapsed tubules lined by scattered Sertoli cells were found.

The brain showed a few small glial foci near the floor of the fourth ventricle as well as a lysis of nuclei and Nissl substance in some of the large ganglion cell centers nearby. Heart and aorta were normal. The lung was hyperemic and the intrapulmonary branches of the pulmonary artery had sub-

COMMENT AND CONCLUSIONS

The bilateral adrenal cortical tumors described are apparently adenocarcinomas derived from adenomas, which, however, have not produced any metastases. They are not tumors of the interrenal type, as the testicular atrophy observed is attributable to a testiculotoxic action of the dye injected, as is evident from a more extensive toxicopathologic study of the effects of excessive doses of this dye introduced into dogs, cats, rabbits, and rats (Hueper and Ichniowski). The toxic action of T-1824 accounts also for the other pathological lesions noted in the various organs. It is not likely that the adrenal tumors found in this particular rabbit were directly or indirectly elicited by the toxic action of the dye, as none of the numerous animals of different species treated similarly showed

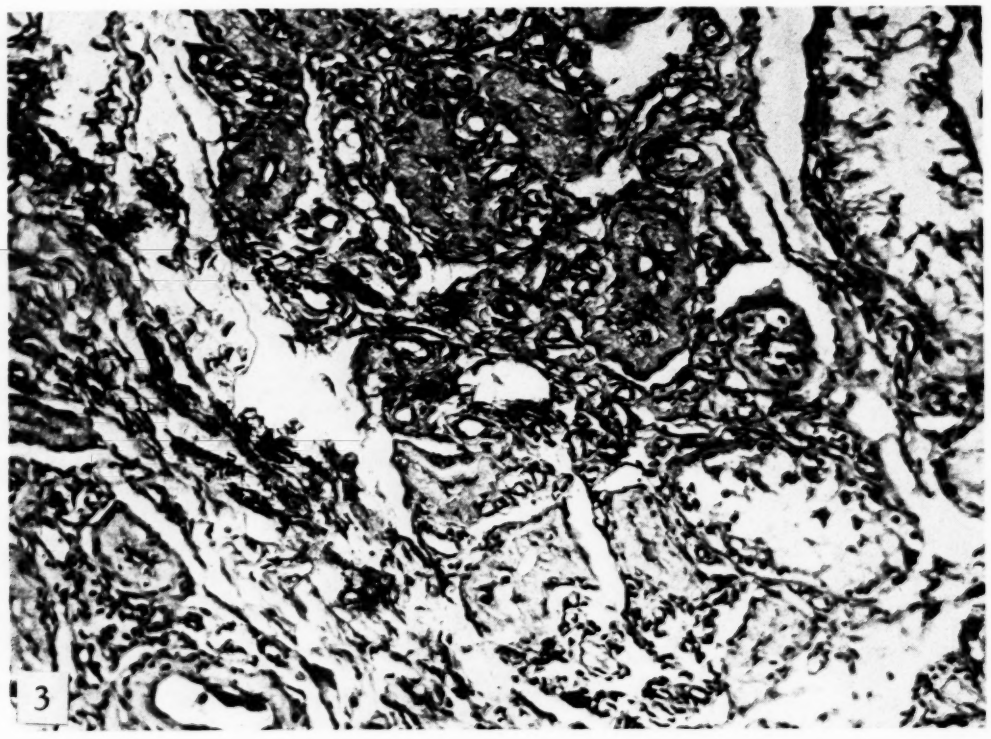

FIG. 3.—Extensive testicular atrophy with collapsed tubules surrounded by thick hyaline sheaths.

any signs of a hyperplasia of the adrenal cortex, unless the time necessary for such a development was in general not sufficiently long.

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Comparative Antifibromatogenic Activity of Progesterone and Related Artificial Steroids*†

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(Received for publication October 25, 1943)

INTRODUCTION

The antifibromatogenic threshold (8), or the quantity of a steroid absorbed daily from a subcutaneously implanted pellet that is sufficient to prevent abdominal fibroids produced in the female guinea pig by α-estradiol, is smallest with progesterone (4, 7, 10). Other steroids having a side chain of two carbons in position 17, as desoxycorticosterone (9, 12) and dehydrocorticosterone (11, 16), also are more active than testosterone. All the four natural steroids mentioned, including testosterone, which so far proved to be antifibromatogenic agents, have a double bond between C_4 and C_5. But dihydrotestosterone, a substance due to the Butenandt and Ruzicka group (3) in which the double bond in ring I is lacking, is as active as testosterone in preventing abdominal fibroids (5, 15). The question arises whether the antifibromatogenic activity of 3-keto-steroids without this double bond might be augmented by adding a side chain of two carbons in position 17. This question is of special interest. The antifibromatogenic order of the active steroids

progesterone > desoxycorticosterone > testosterone

coincides with the progestational order of these steroids (7, 10). The progestational activity of substances without the Δ4-double bond, as for instance Δ5-pregnenolone (13) or ethyl-dihydrotestosterone (6), is only one-tenth or one-twentieth that of progesterone; pregnanedione so far has shown no progestational activity (6). Might pregnanedione, though not progestational, show antifibromatogenic activity?

EXPERIMENTS WITH PROGESTERONE, PREGNANEDIONE, AND ALLO-PREGNANEDIONE

Two pregnanediones were tried. One was prepared by Dr. A. S. Cook from pregnanediol of the urine of pregnant women; m.p., 121° C.[1] The other was allo-pregnanedione, prepared by Dr. Karl Miescher from cholesterol; m.p., 201° C. To facilitate preparation of hard tablets and to slow down the rate of absorption, the "specific" steroid was mixed with cholesterol dissolved in ether; the mixture, containing 40 per cent of the specific steroid and 60 per cent of cholesterol, was dried and triturated. Tablets were prepared that weighed about 30 mgm. each; ½ of a tablet to 2 tablets were subcutaneously implanted in castrated female guinea pigs. For comparison, experiments with synthetic progesterone (prepared by Dr. M. Tausk) also were made; ¼ of a tablet to 1 tablet containing 40 per cent of progesterone and 60 per cent of cholesterol were used for each animal. Fragments of tablets of α-estradiol were implanted simultaneously with those of the steroids mentioned. The animals were sacrificed 90 days after implantation of the tablets.

TABLE I

Mixture of cholesterol with	Duration of experiment, days	Cholesterol: Originally implanted, per cent	Cholesterol: Found, per cent	Specific steroid: Originally implanted, per cent	Specific steroid: Found, per cent
Progesterone	0	60	59		
"	90	60	57	40	42
Pregnanedione	90	60	56		
Allo-pregnanedione	90	33	35		
Δ16-Dehydroprogesterone	90	60	56		

Absorption was calculated from the loss of weight of the tablets cleaned from the fibrous capsule, washed, and dried *in vacuo*. As first shown by Shimkin and White (14), absorption of stilbestrol from tablets containing cholesterol and stilbestrol was not selective; neither was there a selective absorption of steroid hormones in our present work, as shown in Table I.

* This investigation has been aided by a grant from The Rockefeller Foundation, and from The Jane Coffin Childs Memorial Fund for Medical Research. Thanks are due also to Dr. Karl Miescher of Ciba Pharmaceutical Products in Basel, and to Drs. A. S. Cook of Ayerst, McKenna, and Harrison, of Montreal, for different steroids.

† For clarity, we characterized steroids not occurring naturally as *artificial*, in distinction to steroids that are found in the body though used in a *synthetic* form.

[1] Thanks are due to Dr. J. Calvo, of the Laboratory of Physiological Chemistry of the Universidad de Chile for the determination of the melting points, and to Miss N. Pick for technical help.

Cholesterol was precipitated with digitonin from an alcoholic solution of the mixture, washed, and dried. Progesterone was weighed after the elimination of the surplus of digitonin. For details, see Fuenzalida (2). As seen in the table, the percentage of cholesterol and progesterone 90 days after subcutaneous implantation was the same as in the original mixture. Determination of the cholesterol percentage was made in the remaining cholesterol-hormone tablets, with results similar to those obtained with the cholesterol-progesterone tablets.

The fibrous abdominal reaction was classified according to the methods explained in former papers. Results are given in Table II. The antifibromatogenic diagrams (8) are reported in Fig. 1.

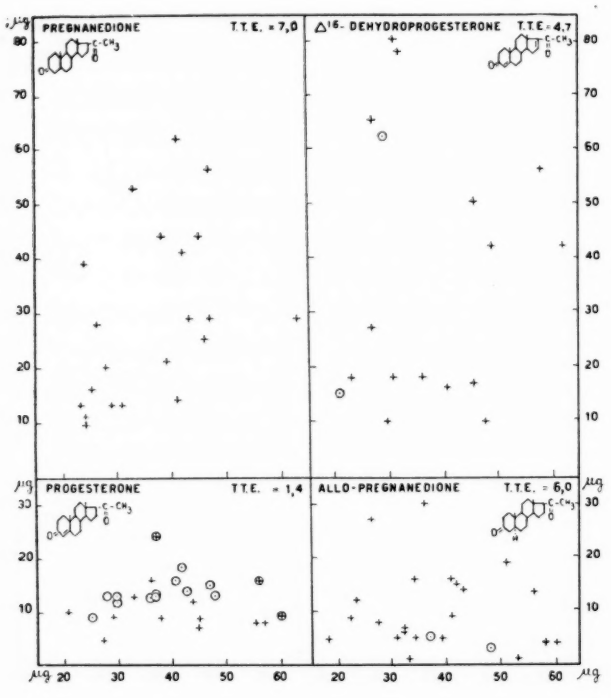

FIG. 1.—Antifibromatogenic diagrams of four different steroids used in a total of 88 castrated female guinea pigs. Quantities of progesterone and other steroids absorbed daily are plotted as ordinates, against estradiol absorbed daily as the abscissa. + = animals with fibroids; ⊙ = animals without fibroids; ⊕ = animals with some fibroid not quite reaching the size of 1 to 1.5 mm. in diameter, or with notable fibrous strands. With more than 10 μgm. of progesterone daily, most animals were without fibroids. The difference between progesterone and allo-pregnanedione is best seen when the figure is covered with a sheet of paper reaching the horizontal division line at 10.

With less than 12 μgm. of progesterone daily, most animals showed abdominal fibroids. With 13 to 24 μgm. daily, fibroids were absent, with the exception of 2 of 14 animals. On the other hand, with pregnanedione abdominal fibroids were present in all animals. The average fibrous tumorous effect (F.T.E.) in animals was even greater with pregnanedione and allo-pregnanedione than with estradiol alone. The difference between the progesterone group on the one hand, and both pregnanedione groups on the other, was statistically highly significant.

In Fig. 2 the fibrous effect is given separately for each animal of Table II. A glance at the figure shows that the difference between the progesterone group with 13 to 24 μgm. daily on the one hand, and both pregnanedione groups on the other, was enormous. We can conclude from the present experiments that 13 μgm. of progesterone were more effective than 60 μgm. of pregnanedione or 30 μgm. of allo-pregnanedione in the prevention of fibroids elicited by estradiol. It seems probable that quantities of progesterone even smaller than 13 μgm. daily were still active; the fibrous effect with 6 to 12 μgm. of progesterone daily was smaller than with estradiol alone. Indeed, in view of the great variation always met in experiments with the fibromatogenic action of estrogens, we do not consider the difference of 1.9 as statistically significant. But the results might be interpreted tentatively to signify that beneath the division line at 13 μgm. of progesterone daily (see Figs. 1 and 2) there is a *transitional zone* in which antifibromatogenic action of progesterone also is present but is less pronounced.

The effect, according to our system of classification, is the sum of four separate values that refer to four different abdominal regions: subserous uterine tumors and those of the parametrium; tumors of the mesosalpinx, or "apical" tumors; tumors of the digestive tract and the abdominal wall; tumors of the spleen. Fibrous strands or small nodules just visible to the naked eye, which might be present in each of these four regions, are classified as 0.5. Thus an animal may show a fibrous effect of 2.0 without a single tumor of 1.5 mm. or more in diameter being present. This drawback in our classification can be corrected by counting the regional values of classes 2 and 3 (tumors of 3 mm. or more in diameter) in the whole group and calculating the incidence of these values for each animal. This incidence (column 9 of Table II) was 2.3 and 1.9 in the pregnanedione groups. On the other hand, there was not a single animal with tumors of class 2 or 3 in the corresponding progesterone group. The great difference is emphasized on the diagrams in Fig. 3. The average fibrous tumorous effect with 13 to 62 μgm. of pregnanedione (F.T.E.=7) in the present series was greater than with estradiol alone (F.T.E.=5.1). An example of one of these animals is given in Fig. 4.

EXPERIMENTS WITH Δ16-DEHYDROPROGESTERONE

One might infer from our results that a steroid whose progestational activity diminishes greatly on

TABLE II: ONE HUNDRED THREE CASTRATED FEMALE GUINEA PIGS INTO WHICH TABLETS OF ESTRADIOL AND TABLETS OF ONE OF THE FOUR FOLLOWING STEROIDS WERE IMPLANTED SUBCUTANEOUSLY: PROGESTERONE, PREGNANEDIONE, ALLO-PREGNANEDIONE, AND Δ16-DEHYDROPROGESTERONE. THE ANIMALS WERE SACRIFICED 90 DAYS AFTER THE SIMULTANEOUS ACTION OF ESTRADIOL AND THE STEROIDS MENTIONED

Group (Series XLVII) (1)		Estradiol per day, μgm. (2)	3-Keto-steroid per day, μgm. (3)	Number of animals: Total (4)	Number of animals: With tumors not smaller than class 1 (5)	Fibrous tumorous effect: Average (6)	Fibrous tumorous effect: Significant difference (7)	Number of tumorous marks of classes 2 and 3: Total (8)	Number of tumorous marks of classes 2 and 3: Per animal (9)
Estradiol alone		16-46	0	15	15	5.1 ± 0.61 †	§ —	25	1.7
Estradiol and:									
I									
Progesterone	(a)	21-60	5-12	12	9 (1) *	3.4 ± 0.66	1.9	12	1.0
"	(b)	28-56	13-24	14	2 (2)	1.4 ± 0.12	—	0	0
II									
Pregnanedione	(a)	24	10-11	2	2	6.0			
"	(b)	23-63	13-62	19	19	7.0 ± 0.51	10.6 ‡	44	2.3
III									
Allo-pregnane-dione	(a)	18-58	1-12	16	14	6.1		32	2.0
"	(b)	23-56	13-30	8	8	6.0 ± 0.94	4.8	15	1.9
IV									
Δ16-Dehydro-progesterone	(a)	30-48	10	2	2	9.5			
"	(b)	21-62	15-80	15	12	4.7 ± 0.71	4.6	21	1.4

* In brackets—with tumors transitional between 0.5 and 1, or with thick fibrous strands classified as 1.

† $\epsilon = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

‡ $\frac{m_{II} - m_{Ib}}{\sqrt{\epsilon_{II}^2 + \epsilon_{Ib}^2}}$

§ Arrow indicates comparison with . . . .

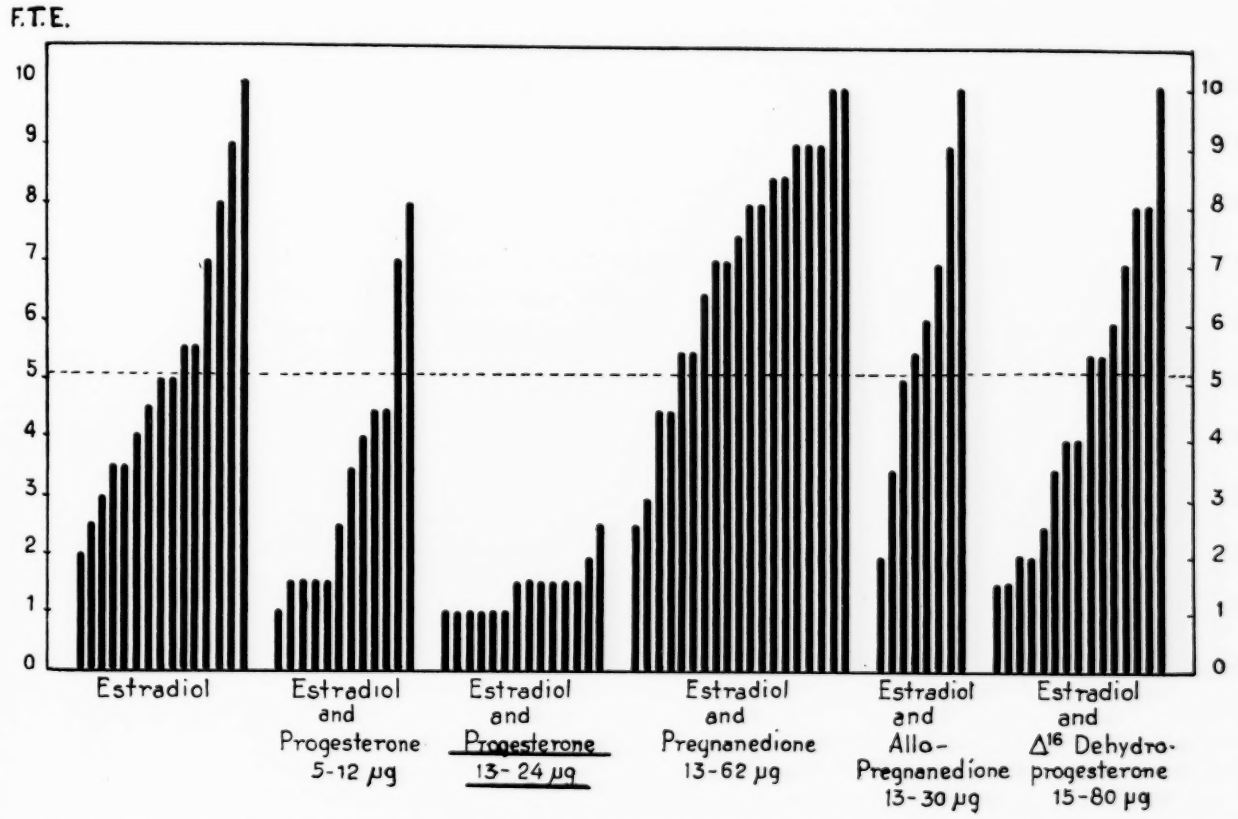

FIG. 2.—Fibrous tumorous effect in the animals of the different groups. Height of the columns indicates the F.T.E. of individual animals, according to our arbitrary scale. Broken line = *average* F.T.E. of the estradiol group. Animals with less than 13 μgm. of artificial steroids are omitted. Fifty per cent of the animals with estradiol alone reach the average F.T.E. = 5 of the group; this is a constant phenomenon in our experiments of three months' duration. This average is reached by more than 50 per cent in the groups simultaneously receiving estradiol and pregnanedione, allo-pregnanedione, or Δ16-dehydroprogesterone. On the other hand, in the estradiol-progesterone group with 13 to 24 μgm. of progesterone daily there was not a single animal that would have reached the average F.T.E. of the estradiol group. The F.T.E. was lowered even with smaller quantities of progesterone: *transitional zone;* see text.

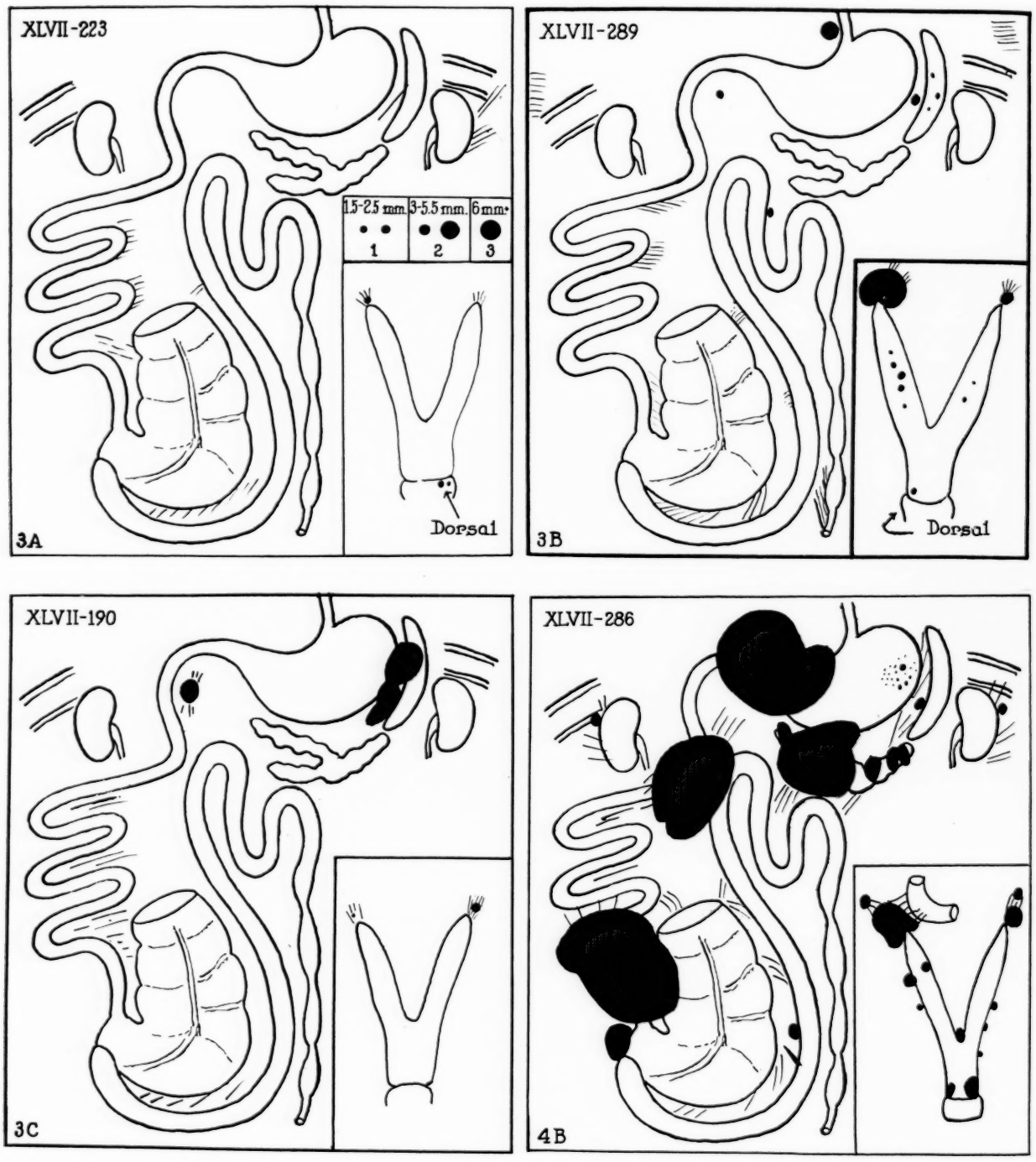

FIG. 3.—A. *Maximal* reaction of castrated female guinea pig with estradiol-progesterone above 12 μgm. of progesterone daily (XLVII, 223). Estradiol daily, 36 μgm.; progesterone daily, 16 μgm. Uter. tum. = 1; apic. tum. = 1; abdom. fibrous strands = 0.5. F.T.E. = 2.5. B. Somewhat more than *average* reaction with estradiol-pregnanedione (XLVII, 289). Estradiol daily, 33 μgm.; pregnanedione daily, 53 μgm. Uter. tum. = 1; apic. tum. = 3; dig. tract and abdom. wall = 3; spleen = 1. F.T.E. = 8. C. Somewhat more than *average* reaction with estradiol-allo-pregnanedione (XLVII, 190). Estradiol daily, 34 μgm.; allo-pregnanedione daily, 16 μgm. Apic. tum. = 1; dig. tract = 3. Spleen = 3, F.T.E. = 7. All diagrams, including the scale in Fig. 3A, have been reduced to half the natural size.

account of hydrogenation (lack of the Δ4-double bond) or of some other chemical changes, loses most of its antifibromatogenic activity; or, in other words, that antifibromatogenic activity is concomitant with progestational activity. For this reason experiments with dehydroprogesterones are of special interest. We have used Δ16-dehydroprogesterone, prepared by Dr. Karl Miescher. This compound, if it be progestational at all, is several times less active than progesterone. It revealed no progestational action in the rabbit test with 5 mgm. (1). In our work, 80 μgm. of Δ16-dehydroprogesterone daily showed no antifibromatogenic action (see Table II and also Figs. 1 and 2).

FIG. 4.—Four large abdominal fibroids in castrated female guinea pig with absorption of 33 μgm. of estradiol and of 53 μgm. of pregnanedione daily. A. One large and several smaller tumors of the great curvature of the stomach and epiploon, and nearby to the right, tumor between jejunum and ascending colon. Above these tumors: large tumor of the ventral abdominal wall, near the diaphragm. Beneath: large tumor between ileum and cecum. B. Diagram of the same case.

Other Toxic Actions of Estradiol

Other known toxic actions of estradiol are genital bleeding and massive uterine growth. Results with progesterone and the three artificial steroids are given in Table III.

The small quantities of progesterone that were sufficient to prevent abdominal fibroids (see Table II) usually prevented massive uterine growth but not in all animals. In several cases there was even necrosis of the uterine wall induced by a prolonged administration of estrogens. Uterine bleeding never occurred.

On the contrary, uterine growth and uterine bleeding were not prevented by the three artificial steroids.

Experiments with Larger Quantities of the Three Steroids

As seen from Table II and from Figs. 1 and 2, the three steroids mentioned were not antifibromatogenic even with quantities 2 to 6 times greater than the antifibromatogenic threshold quantity of progesterone.

TABLE III

Group	Number of animals	Uterine weight		Number of animals with	
		Average gm.	Range gm.	Uterine bleeding	Necrosis of uterine wall
Estradiol alone	15	5.7	3.0-12.0	4	3
Estradiol and:					
Progesterone					
5-12 μgm.	12	3.2	1.9-5.2	0	0
13-24 μgm.	13	3.0	1.7-5.1	0	3
Pregnanedione					
13-62 μgm.	17	7.0	4.2-12.2	7	7
Allo-pregnanedione					
13-30 μgm.	8	4.8	2.5-9.2	1	3
Δ16-Dehydroprogesterone					
15-80 μgm.	15	5.0	3.0-10.8	4	1

The question arises whether an antifibromatogenic activity might be observed with quantities exceeding those mentioned above.

Table IV refers to 11 experiments in which quantities 10 to 30 times greater than those of the progesterone threshold were used. Comparison of the results given by 200 to 400 μgm. of pregnanedione daily with those elicited by smaller quantities (Table II) suggests that with about 300 μgm. daily there might be an antifibromatogenic action; this would give an antifibromatogenic threshold about 25 times greater than that of progesterone.

Experiments with large quantities of allo-pregnanedione and Δ16-dehydroprogesterone gave results corresponding to what has been described above as the transitional zone with 5 to 12 μgm. of progesterone, though experiments with large quantities of the two steroids mentioned were not numerous enough to allow for conclusions. However, these experiments suggest that the antifibromatogenic threshold of these steroids would be about 15 to 20 times greater than that of progesterone.

Whereas our results show that antifibromatogenic

activity is often concomitant with progestational activity, they show also that with huge quantities of a steroid hitherto not known to be progestational some antifibromatogenic action can be obtained. On the other hand, there is the possibility that these steroids hitherto considered as non-progestational might reveal progestational activity if sufficient quantities were used. As already mentioned, progestational activity of certain steroids, according to different authorities (6, 13), is dependent upon quantities 25 to 50 times greater than those of progesterone.

Since pregnanedione so far is not known to be progestational, our findings suggest that antifibromatogenic activity is concomitant to a certain extent with the progestational one.

This suggestion was corroborated by experiments with the non-progestational Δ16-dehydroprogesterone, which revealed no antifibromatogenic activity even when quantities were absorbed that were 6 times greater than the antifibromatogenic threshold of progesterone.

When quantities were absorbed that were 10 to 30 times greater than the antifibromatogenic threshold of progesterone, the three nonprogestational steroids revealed some antifibromatogenic action comparable with that observed in the transitional zone with progesterone.

TABLE IV: ELEVEN CASTRATED FEMALE GUINEA PIGS WITH SUBCUTANEOUSLY IMPLANTED TABLETS OF α-ESTRADIOL AND DIFFERENT 3-KETO-STEROIDS. DURATION OF THE EXPERIMENTS 90 TO 92 DAYS

Group (Series XLVII)	Estradiol per day, μgm.	3-Keto-steroid per day, μgm.	Total fibrous tumorous effect (F.T.E.)	Tumorous class: Uterine subserous	Uterine apical	Digestive tract and parietal	Splenic	
Pregnanedione *								
No. 314	34	198	7.0	0	3 t.c.	2 ms.	2	
No. 313	27	257	6.0	0	3	3	0	uter. bleed.
No. 317	33	276	2.5	1 s.s.v.	0.5 f.	0.5 f.s.	0.5 f.s.	
No. 311	41	324	1.5	0	0.5 f.	0.5 f	0.5 s.f.	uter. bleed.
No. 315	38	404	2.0	0	1	0.5 f.s.	0.5 f.s.	
Allo-pregnanedione *								
No. 306	30	53	1.5	0	0.5 f.	0.5 f.	0.5 f.	
No. 299	21	110	2.5	0.5 s.v.	0.5 f.	1 ms.	0.5 s.	
No. 305	35	146	1.5	0.5 s.v.	0.5 f.	0.5 f.s.	0	
No. 304	42	152	3.5	0	2 t.c.	0.5 f.s.	1. s.s.	
Δ16-Dehydroprogesterone *								
No. 325	34	160	3.5	0	1	2 cr.	0.5 f.s.	
No. 324	44	240	1.0	0	0	0.5 f.	0.5 f.	

* Tablets containing 67% of pregnanedione and 33% of cholesterol; or pure allo-pregnanedione; or 40% of Δ16-dehydroprogesterone and 60% of cholesterol. *Several* tablets have to be implanted when absorption of quantities so considerable is intended. At necropsy tablets are sometimes found in a mushy condition or they are partly lacking; similar experiments have been omitted from Table II.

f. = fibrous strands; s. = accumulation of small nodules or tumorous seed; s.s. = very pronounced tumorous seed; s.v. = seed on vaginal peritoneum; t.c. = tumorous thickening of tubal cyst; ms. = mesenteric tumor; cr. = tumor near cardia.

SUMMARY

The preventive action of synthetic progesterone against abdominal fibroids elicited by α-estradiol in the guinea pig was obvious when only 13 to 24 μgm. of the 3-keto-steroid were absorbed daily from a subcutaneously implanted tablet.

The antifibromatogenic quantity of progesterone was less than the quantity of the fibromatogenic estrogen absorbed simultaneously.

There was still some preventive action with quantities of progesterone smaller than 13 μgm. daily (*transitional zone*).

On the contrary, quantities of pregnanedione or of allo-pregnanedione equal to antifibromatogenic quantities of synthetic progesterone, or quantities several times greater than the latter, were unable to prevent abdominal fibroids elicited by estradiol.

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On the Antifibromatogenic Activity of Synthetic Progesterone in Experiments with the 17-Caprylic and Dipropionic Esters of α-Estradiol*

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(Received for publication October 25, 1943)

INTRODUCTION

Through the work of Biskind (2), Segaloff and Nelson (18), and of Lipschütz and his students (1, 3, 7, 8) considerable evidence has been accumulated in favor of greater resistance of *esterified* estrogens against hepatic inactivation when compared with *free* estrogens. Esterification protects estradiol against inactivation in the liver, though this protection is a limited one.

The question arises whether esterification protects estradiol also against the antagonistic action of progesterone and other steroids in preventing fibroids elicited by estrogens. This question is of considerable interest. Should esterification prove unable to give any protection at all against the antifibromatogenic action of steroids, this would be a new proof in favor of the assumption that antagonizing of one steroid by another is an event *per se* independent of hepatic events. In other words, antagonization of steroids might then be considered as representing a special means of adjustment of the steroid balance supposed to be one of the endocrine aspects of antitumorous autodefense in the body (9, 10).

In former experiments with the simultaneous injection of the benzoic ester of estradiol and of progesterone, abdominal fibroids were prevented (13, 14, 17). Huge quantities of progesterone were used; they were 150 times greater than the quantity of the esterified estradiol administered. In the meantime, however, it has been found in experiments with simultaneous implantation of tablets of estradiol and progesterone that fibroids can be prevented with a quantity of progesterone even smaller than the amount of free estradiol simultaneously absorbed (4). It was thought that the question whether esterification protects the estrogen against antagonization by other steroids might best be studied by establishing the quantity of progesterone that would still prevent fibroids induced by subcutaneously implanted tablets of a fibromatogenic ester of estradiol as potent as the 17-caprylate (11) or the dipropionic ester, which has been shown to be especially resistant against intrahepatic inactivation (18).

* This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research and The Rockefeller Foundation. Thanks are due to Dr. Karl Miescher and to Dr. C. Oppenheimer, Ciba Pharmaceutical Products, for furnishing steroids.

EXPERIMENTS

Results with 54 castrated female guinea pigs are given in Table I. Each of these animals had a subcutaneously implanted tablet, or fragment of tablet, of esterified estradiol on one side of the body, and a tablet of progesterone on the other side. All these experiments lasted from 61 to 65 days, with the exception of three that lasted from 67 to 68 days. The tablets of the dipropionate were 2 to 11 mgm.; those of the caprylate were 20 to 50 mgm. Absorption from subcutaneously implanted tablets of the caprylic ester is about 5 times less than that from tablets of the dipropionic ester (15); for this reason the surface has to be greater with the caprylic ester so as to guarantee absorption of equal quantities of both esters. The tablets, or fragments, of progesterone were 8 to 15 mgm. in the series with the caprylic ester. In most of the experiments with the dipropionic ester, mixed progesterone-cholesterol tablets were used, so as to slow down absorption and thus allow absorption of small quantities of progesterone. The tablets contained 40 per cent of progesterone and 60 per cent of cholesterol. According to unpublished work of Fuenzalida, absorption of progesterone from similar tablets is not selective; in our experiments Fuenzalida found no increase of the cholesterol percentage in the course of 60 days. The mixed tablets or fragments of these weighed 8 mgm. and upwards.

The daily absorption was calculated from the loss of weight of the tablet dried *in vacuo*. The fibrous tumorous effect (F.T.E.) was classified according to rules explained in former papers.

Antifibromatogenic Action of Progesterone Against the 17-Caprylic Ester of Estradiol

As seen in Table I, the antagonistic action of progesterone was evident when the 17-caprylic ester

of α-estradiol was used as a fibromatogenic agent. There was not a single animal with tumors of 1.5 mm. or more in diameter when progesterone was acting simultaneously with this ester. The average recorded fibrous or tumorous effect of 1.5 in the caprylate-progesterone group was due exclusively to fibrous peritoneal strands or to small nodules just visible to the naked eye. Increase of uterine weight also was greatly lowered and there was no genital bleeding.

The antagonizing action of progesterone was still obvious when no more than 58 μgm. were absorbed daily. A graphic representation of the results with 58 μgm. of progesterone is given in Figs. 1 and 2. The ratio between estradiol and progesterone absorbed daily was 1:1.5 in this latter case and 1:1.4 in another case. On the other hand, the same quantity of estradiol when present alone in the body produced tumors in 9 of 10 animals (Table I). Individual results with 10 animals receiving the caprylate of estradiol alone and 10 animals simultaneously receiving progesterone are given in Fig. 3. Not a single animal of the caprylate-progesterone group (B) reached the average F.T.E. of the caprylate group (A).

From what we know from our extensive work with the antifibromatogenic threshold of progesterone acting against free estradiol, we must infer that the antifibromatogenic threshold of progesterone with the 17-caprylic ester is smaller than 58 μgm. With free estradiol and with smaller quantities of progesterone the antifibromatogenic diagram showed a *transitional zone* in which the fibrous tumoral reaction, though present, was seemingly less pronounced than in the total absence of progesterone, as in Fig. 1 of the preceding paper, beneath the division line at 13 μgm. But this transitional zone was not yet reached in our present work with the caprylic ester of estradiol and this suggests that with quantities smaller than 58 μgm. of progesterone there will be prevention of fibroids elicited by the caprylic ester.

TABLE I:* FIFTY-FOUR CASTRATED FEMALE GUINEA PIGS WITH SUBCUTANEOUSLY IMPLANTED TABLETS OF ESTERIFIED ESTRADIOL AND PROGESTERONE. DURATION OF THE EXPERIMENTS 61 TO 68 DAYS

Group		Absorbed per day		Number of animals		Average F.T.E.	Animals with genital bleeding	Uterine weight, gm.
		Estradiol †, μgm.	Progesterone, μgm.	Total	With tumors			
Caprylate	A	32 (13-59) ‡	0	**10**	**9**	3.3	6	4.3 (2.5-10.5)
Caprylate and progesterone	B	32 (17-60)	**58**-120	**10**	**0**	1.5	0	2.1 (1.5-3.2)
Dipropionate	A	23 (11-31)	0	10	9	4.5	2	4.7 (3.1-10.2)
Dipropionate and progesterone	B	27 (12-45)	55-140 §	15	1 ¶	1.0	0	2.0 (1.2-3.7)
"	C	21 (11-31)	**28**-49 ‖	**5**	**0**	1.0	0	1.5 (0.9-2.2)
"	D	29 (23-36)	6-11 ‖	4	3	2.8	0	2.5 (1.8-3.7)

* Selected from the thesis presented for the degree of M.D. by J. Grismali (5) in the Universidad de Chile. Minor changes have been made in the evaluation of F.T.E. (fibrous tumorous effect) in various animals.

† Always calculated as free estradiol.

‡ Figures in brackets indicate range.

§ Five experiments with pure progesterone, 102 to 140 μgm. daily. The remaining experiments with tablets containing 40 per cent of progesterone and 60 per cent of cholesterol.

‖ All experiments with tablets mixed with cholesterol.

¶ With 116 μgm. of progesterone and 42 μgm. of estradiol daily.

ANTIFIBROMATOGENIC ACTION OF PROGESTERONE AGAINST THE DIPROPIONIC ESTER OF ESTRADIOL

In experiments in which the dipropionic ester of α-estradiol (Table I) was used as a fibromatogenic agent, there were tumors in only 1 of 20 animals receiving 28 to 140 μgm. of progesterone, whereas with the dipropionic ester present alone in the body 9 of 10 animals had tumors (Table I). The smallest ratio between estradiol and progesterone, in cases ranging between 28 and 49 micrograms of the latter, was 1:1.4 (Figs. 4 and 5).

In the small group with minute quantities of progesterone, 6 to 11 μgm. daily, uterine and extragenital fibroids were not prevented (Table I and Fig. 6), though the fibrous reaction was not very pronounced. With these small quantities of progesterone acting against the dipropionic ester of estradiol, the

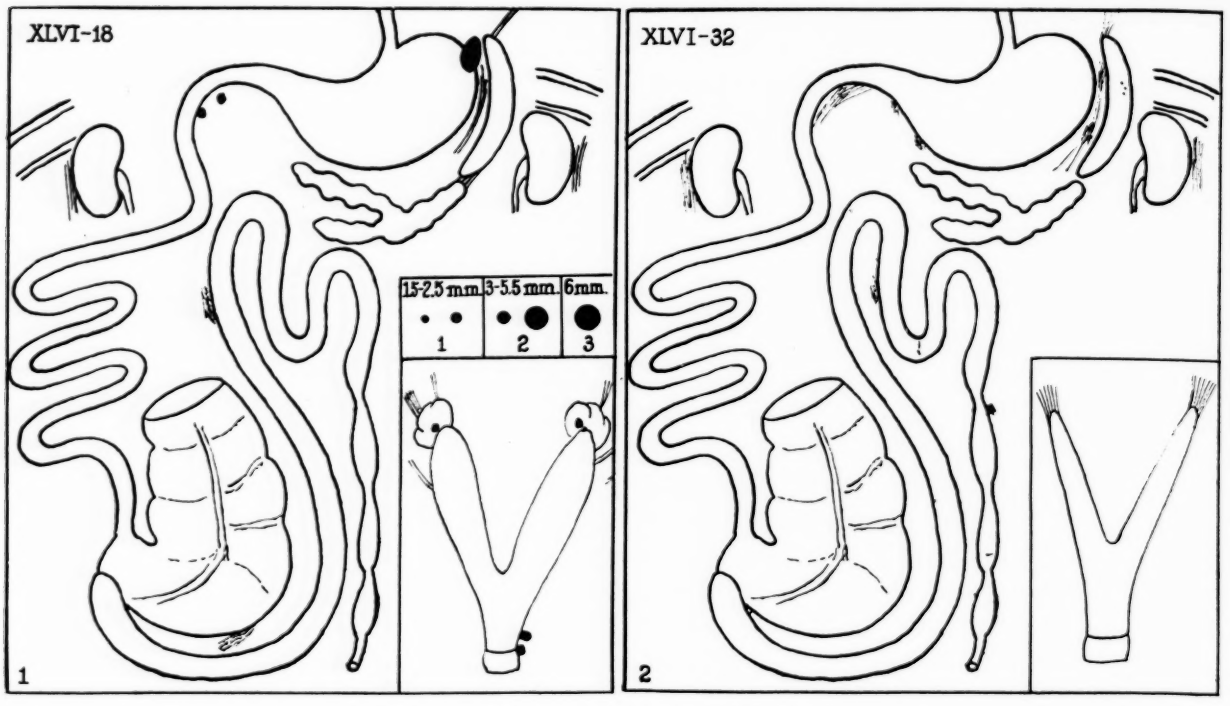

FIG. 1.—Caprylic ester of estradiol, 61 days (XLVI-18). Estradiol daily, 13 μgm. Subserous uterine tumors near the vagina = 1; apical tum. = 1; mesent. tum. of the duodenum = 1; splenic tum. = 2. F.T.E. = 5. Weight of uterus, 3.6 gm.

FIG. 2.—Caprylate of estradiol and progesterone, 64 days (XLVI-32). Estradiol daily, 38 μgm.; progesterone daily, 58 μgm. No tumors; only fibrous strands in the abdominal cavity and small nodules on the surface of the spleen. F.T.E. = 2. *Maximal reaction of this series.* Weight of uterus, 2.8 gm. Diagrams of Figs. 1 and 2, including the scale in Fig. 1, have been reduced to half the natural size.

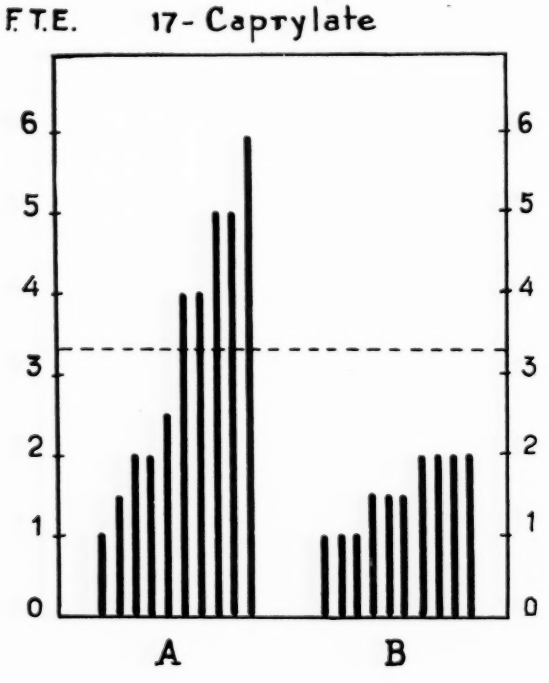

FIG. 3.—F.T.E. of 10 animals receiving the 17-caprylic ester of estradiol (A) and of 10 simultaneously receiving the 17-caprylic ester and progesterone (B). See Table I. In the B group there was not a single animal that would have reached the average F.T.E. of the A group (broken line).

transitional zone seems to have been reached; this zone coincides with that of similar quantities of progesterone acting against free estradiol. See the antifibromatogenic diagram of progesterone against free estradiol in the preceding paper (12). The antifibromatogenic threshold of progesterone, acting against the dipropionic ester of estradiol, was supposedly between 11 and 28 μgm. daily in the present experiments. This makes it very likely that the antifibromatogenic action of progesterone against the dipropionic ester occurs under similar quantitative conditions as its action against the free estrogenic hormone.

The preventive action of progesterone in the C group, with 28 to 49 μgm. daily, and even in the D group, with only 6 to 11 μgm. daily, also was obvious with reference to the uterine weight, as seen from Table I.

DISCUSSION

Our results with the preventive action of progesterone against the fibromatogenic action of esterified estradiol give no support to the assumption that esterification protects against the antifibromatogenic action of progesterone. Accordingly, when referring to the antagonizing of the estrogen by another steroid such as progesterone and desoxycorticosterone on the one hand, and to the metabolic fate of the estrogen in the

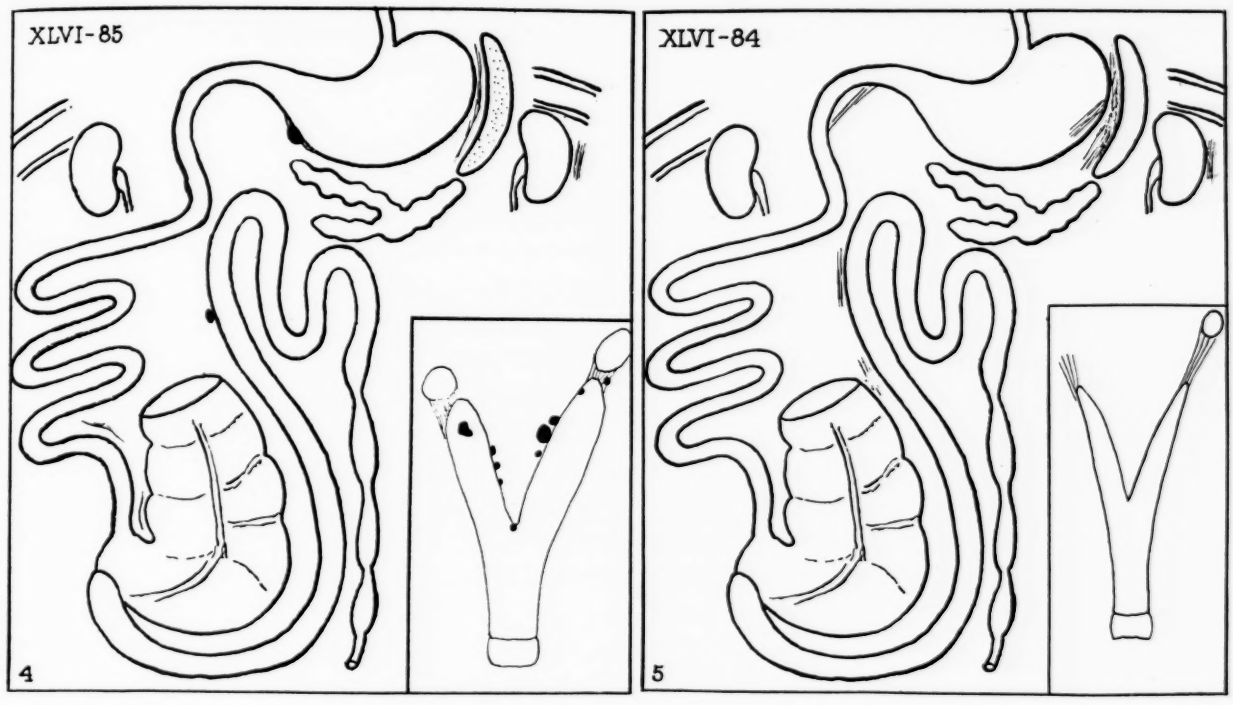

FIG. 4.—Dipropionic ester of estradiol, 67 days (XLVI-85). Estradiol daily, 14 μgm. Parametric and subserous tumors of the uterus = 2; apical tum. = 0.5; tum. of the epiploon and mesocolon = 1; small nodules on the surface of the spleen = 0.5. F.T.E. = 4. Weight of uterus, 10 gm.

FIG. 5.—Dipropionic ester of estradiol and progesterone, 68 days (XLVI-84). Estradiol daily, 14 μgm.; progesterone daily, 32 μgm. No tumors, only fibrous strands. F.T.E. = 1.5. *Maximal reaction of this series.* Weight of uterus, 1.6 gm. Diagrams of Figs. 3 and 4, including the scale in Fig. 1 (above) have been reduced to half the natural size.

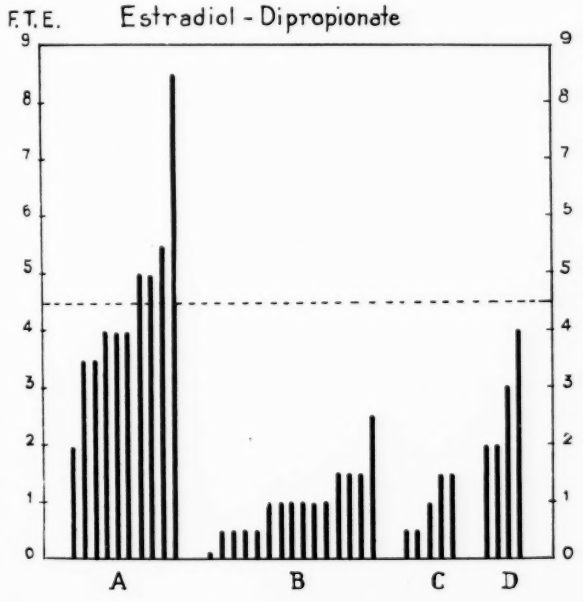

FIG. 6.—F.T.E. of 10 animals receiving the dipropionic ester of estradiol (A) and of 24 simultaneously receiving the dipropionic ester and progesterone (B, C, D). See Table I. No animal in B and C (55 to 140 μgm. and 28 to 49 μgm. of progesterone) approaches the average of the A group (broken line). In the D group (6 to 11 μgm.) the transitional zone is seemingly reached as with similar quantities of progesterone acting against free estradiol.

liver on the other, we are dealing with two *different* aspects of the antitumorous autodefense of the body (9, 10). We are dealing also with two different *sites* at which the toxic or tumor-producing agent is counteracted. Our actual results make it highly probable that the antagonistic steroid acts as a desensitizer, *i.e.*, renders the tissue incapable of responding to the estrogenic stimulus, as was supposed many years ago on the basis of our findings with experimental hermaphroditism produced by transplantation of the ovary into partially castrated male guinea pigs (6, 16).

SUMMARY

The fibromatogenic action of subcutaneously implanted tablets of the 17-caprylic ester and of the dipropionic ester of α-estradiol was prevented with quantities of progesterone not far from, or identical with, those that are necessary to antagonize the fibromatogenic action of free α-estradiol (antifibromatogenic threshold).

From these findings the conclusion can be drawn that esterification, though relatively protective against the inactivating action of the liver, does not confer protection against the antagonizing action of progesterone.

Our results suggest that the antagonistic action of this steroid against estradiol is effected not by inter-

fering in the inactivation of estrogens in the liver but by rendering the reacting tissue unable to respond to the action of estrogens.

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Phenomenon of Local Skin Reactivity to *Serratia marcescens* (*B. Prodigiosus*)

Immunological Relationships between *Serratia marcescens* Culture Filtrates and Shear Polysaccharide

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(Received for publication October 2, 1943)

Bacterial filtrates capable of producing the phenomenon of local tissue reactivity also elicit hemorrhagic necrosis and regression of transplantable malignant tumors. The destruction of the tumors is obtained by a single intravenous injection, no preparatory intratumoral injection being necessary (25a, 11, 26, 7, 3, 23, 10, and others). There exists considerable evidence that the same bacterial substances are responsible for both the phenomenon-producing and tumor-destroying potencies. The assumption is based on a series of observations by various authors previously reviewed in detail (25a). Briefly, the concentration of the phenomenon-producing factors in bacterial filtrates parallels closely the tumor-destroying potency. Bacterial filtrates devoid of the phenomenon-producing factors, and many toxic nonbacterial substances (including colloidal gold and *crotalus adenamentus,* strongly poisonous to blood capillaries) incapable of eliciting the phenomenon also lack the tumor-destroying property. Shear and Andervont (23) concentrated and purified active filtrates of *B. coli* cultures. With the purified fraction the phenomenon in the rabbit was obtained (2) with 0.5 μgm., which was close to the amount of 0.3 μgm. effective in elicitation of hemorrhage in a mouse sarcoma. Extending these investigations Shear and his coworkers (24, 12) attained a still greater purification and concentration of the agent, which produces hemorrhage and necrosis in tumors, in *Serratia marcescens* culture filtrates by the use of chemical methods. A preparation considered by the authors a polysaccharide and capable of elicting hemorrhage in mouse sarcomas in a dose of 0.1 μgm. was thus obtained. Its phenomenon-producing potency was not determined. The material having kindly been made available by Dr. M. J. Shear to the author of this paper, an opportunity was afforded to study further the relationship between the tumor-destroying and phenomenon-producing potencies of bacterial substances, as follows:

1. The phenomenon-producing titers of culture filtrates and Shear polysaccharide from *Serratia marcescens* were determined quantitatively.

(2) The active principles of the phenomenon of local tissue reactivity being clearly antigenic, immunological relationships between the culture filtrates and Shear polysaccharide were studied by means of cross neutralization and cross precipitation reactions.

EXPERIMENTAL

Materials.—The strain of *Serratia marcescens* used by Shear and his coworkers (24, 12) for preparation of the active polysaccharide was maintained in this laboratory on plain agar slants incubated at room temperature and transplanted twice a week.

For preparation of toxin, a few loopfuls of a 72 hour old plain agar culture were sealed into a simple synthetic medium (24). The latter was prepared by dissolving in a liter of distilled water KH_2PO_4, 5.0 gm.; NH_4Cl, 0.5 gm.; NH_2SO_4, 0.5 gm.; and glucose, 2.5 gm., and sterilizing the solution at 10 pounds' pressure for 10 minutes. The final pH was 4.5-4.6. The inoculated medium was then incubated at room temperature for a period of 4 days and filtered through a Mandler kaolin filter #7 under negative pressure. Twenty-four hours later the filtrate was refiltered through a Mandler #11 candle and a merthiolate solution (1:1000) was added to make a final concentration of 1:20,000.

Two batches of the active polysaccharide supplied by Doctor Shear under the designation P1 and P2 were trypsin digested, purified lyophilized preparations (12) capable of producing hemorrhage in mouse sarcomas in a dose of 0.1 mgm. The necessary amounts of the material were dissolved in normal saline to a concentration of 2.5 mgm. per ml., filtered through a Mandler #7 candle and preserved by means of merthiolate in a final concentration 1:20,000. The solution was stored in the refrigerator. No decline in potency was observed during the several weeks of use of each solution.

The toxin used for preparation of the skin of rabbits about to be described consisted of a mixture of several batches of "agar washings" culture filtrates of meningococcus, strain 44B, Group III, of ascertained potency for the elicitation of the phenomenon of local skin reactivity. The preparation of this toxin was previously described in detail (25b).

I. Phenomenon of Local Skin Reactivity to *Serratia marcescens:* Quantitative Titrations of Culture Filtrates and Shear Polysaccharide

In order to obtain accurate, quantitative comparison of the various preparations studied the rabbits were each prepared by a single intradermal injection of 0.25 ml. of the same batch of meningococcus toxin diluted 1:3. The materials for study, variously diluted, were tested by single intravenous injections in doses calculated per kilogram of body weight. Two rabbits were used for each dilution. The titration method of choice previously described (25c) yielded the following "end-point" titers of the provocative factors of the materials:

The batch of the *Serratia marcescens* culture filtrate used in the experiments about to be described contained 2.5 reacting units per milliliter. A portion of the filtrate was dialyzed against distilled water for several days and then lyophilized. The powder was dissolved in normal saline in an amount equal to one-fifth of the original volume. On titration 0.14 ml. of the concentrate were found to contain one reacting unit per ml.; *i.e.,* a 2.86-fold concentration of reacting potency. The concentration obtained permitted the use of sufficiently large amounts necessary for the "end-point" neutralization experiments described below.

Both P1 and P2 Shear polysaccharides yielded the same results, the minimal provocative dose being 8 μgm. per kg. of body weight.

II. Preparation of Immune Sera

Large rabbits were used preferably. The immunization procedure employed is summarized in Table I.

Surviving rabbits that developed strongly positive reactions of the phenomenon of local skin reactivity were advantageously used for further immunization. Six to 7 days following the intravenous injection a series of intramuscular injections was given at intervals of a few days between the injections. The method was somewhat varied, however. Thus rabbits that yielded sera III and V received only intramuscular injections. In the rabbit of serum IV a course of six intramuscular injections gave antibodies of low potency. One intravenous injection following a rest of 9 days was sufficient to raise substantially the titer of antibodies. Some animals died before the course was completed. Others produced sera of low potency or devoid of antibodies. The protocols of the latter animals are not included in this paper.

Table I: Immunization of Rabbits

Animal designation	Injections				Interval in days between injections						Bleedings		
	Material	Dose	Route	Number of injection	1-2	2-3	3-4	4-5	5-6	6-7	Days between last injection and bleeding	Number of injections preceding bleeding	Serum designation
I	P2-Shear polysacchar.	50γ	intrav.	one	6	3	5	3	5		4	6	I
	" "	500γ	intramusc.	five									
II	" "	12.5γ	intrav.	one	7	3	3	3	5	5	5	6	IIa
	" "	500γ	intramusc.	six							5	7	IIb
III	" "	500γ	intramusc.	six	4	3	5	2	5		5	6	III
IV	" "	500γ	intramusc.	six	4	3	5	2	4	9	5	7	IV
	" "	500γ	intrav.	one									
V	*S. marcescens* culture filtrate	2.4 cc.	intramusc.	six	2	4	3	4	3		5	6	V

The sera described in Table I were employed for the following studies on the antigenic relationships between the *Serratia marcescens* culture filtrates and Shear polysaccharide.

III. Precipitation Reactions of *Serratia marcescens* and Shear Polysaccharide

The "ring" test was used. In trial experiments the temperature and duration of incubation period were varied. It was found that most abundant precipitation was obtained when the tubes were first left at room temperature for 1 hour and then transferred to a water bath at 37° C. for the same period of time. On further incubation in the water bath or when left in the refrigerator overnight, the precipitates tended to dissolve in part or settle to the bottom of the tubes and thus confuse the reading. Precipitation tests were carried out with each serum in three steps, namely: (a) various amounts of the precipitinogen were tested

against a constant amount of serum (usually 0.25 ml. of undiluted serum); (b) the concentration of the precipitinogen showing maximum precipitation was then titrated against variable amounts of serum; (c) the optimum amount of serum found in (b) was finally titrated against variable amounts of precipitinogen. In this manner the "prozone" effect was frequently avoided. The results of the final titrations are summarized in Table II.

As may be seen from Table II, the culture filtrates and Shear polysaccharide of *Serratia marcescens* are notably antigenic. There were obtained potent precipitating antisera giving reactions with minute quantities of homologous antigens; *i.e.,* the smallest reacting amounts of the filtrate and the concentrate being 12.5 μgm. and 0.005 ml. respectively. The cross precipitation reactions recorded indicated a definite antigenic relationship between the culture filtrates and the polysaccharide. Furthermore, a certain fact should be pointed out. The antifiltrate serum contained antibodies of equally high titer for the homologous filtrate and the heterologous polysaccharide. Thus the antifiltrate serum V gave precipitation with polysaccharide in the same titer as the anticoncentrate serum III. On the other hand, the anticoncentrate serum III gave precipitation of a lower titer with the filtrate than the homologous serum V. Cross absorption experiments were not carried out for lack of some of the sera. The cross precipitation tests recorded suggest, however, that the culture filtrates of *Serratia marcescens* possess a broader and possibly higher antigenicity than the Shear polysaccharide. This point will be taken up in greater detail in the discussion.

TABLE II: CROSS PRECIPITATION REACTION OF SHEAR POLYSACCHARIDE P2 AND *Serratia Marcescens* CULTURE FILTRATE

Serum designation	Precipitin: Immunizing antigen	Precipitin: Amount	Precipitinogen	Precipitation Reaction, Various amounts of precipitinogen: Amt.	Rea.	Amt.	Rea.	Amt.	Rea.	Amt.	Rea.	Amt.	Rea.	Amt.	Rea.
IIa	P2-Shear polysacchar.	0.4 cc.	P2-Shear polysacchar.	250γ	1+	125γ	1+	62.5γ	1+	25γ	±				
IIb	" "	0.4 cc.	" "	250γ	3+	125γ	2+	62.5γ	1+	25γ	1+				
III	" "	0.5 cc.	" "	250γ	4+										
III	" "	0.4 cc.	" "	250γ	3+	125γ	3+	62.5γ	2+	25γ	1+	12.5γ	±		
III	" "	0.25 cc.	" "	1500γ	3+	750γ	3+	187.5γ	3+	93.8γ	2+	46.9γ	1+	23.5γ	±
III	" "	0.5 cc.	*S. marcescens* culture filtrate	0.3 cc.	3+										
III	" "	0.4 cc.	" "	0.3 cc.	2+	0.2 cc.	1+	0.1 cc.	1+	0.05 cc.	−				
V	*S. marcescens* culture filtrate	0.4 cc.	" "	0.2 cc.	4+	0.04 cc.	3+	0.01 cc.	2+	0.005 cc.	1+				
V	" "	0.4 cc.	" "	250γ	3+	125γ	2+	62.5γ	2+	25γ	1+	12.5γ	±		

Amt. = amount.
Rea. = reading.

IV. SERUM NEUTRALIZATION OF THE PROVOCATIVE FACTORS OF THE PHENOMENON OF LOCAL SKIN REACTIVITY TO SERRATIA MARCESCENS

The neutralizing potency of the immune sera described was measured by determining the ability of the sera to inactivate the provocative factors of the phenomenon of local skin reactivity. The tests were carried out in a manner already described (25d), as follows:

The skin of each rabbit was prepared by a single injection of the same meningococcus filtrate as was used for the titration of the provocative factors (Section I), in a dose of 0.25 ml. of the filtrate diluted 1:3. Twenty-two to 24 hours later a single intravenous injection of the filtrate alone, suitably diluted, or of a mixture prepared with the immune serum, was made. The mixtures prepared on the morning of the experiments, as well as the filtrate alone, were incubated in the water bath at 37° C. for 1 hour. The reactions were read 4½ and 24 hours after the intravenous injection. In the "negative" rabbits no reactions were seen. "Positive" animals developed typical reactions within 4½ hours following the intravenous injections, whilst in the "doubtful" ones there were seen mild punctiform hemorrhagic areas of dark red appearance, usually only 22 to 24 hours after the provocative injection. By the use of the above method of recording of the reactions, reliable results could be obtained when each mixture was tested in two rabbits. When both animals showed no reactions the neutralization was considered complete (CN). With both rabbits developing doubtful reactions; or one rabbit remaining negative, while the mate showed a positive reaction, the neutralization was recorded as incomplete (IN) and usually occurred near the end-point of the titration. Obviously, no neutralization (NN) occurred when both rabbits developed typical reactions within 4½ hours following the intravenous injection. The amount of each ingredient of the mixtures tested is given in Table III.

As may be seen from Table III, the active principles of the phenomenon of local skin reactivity in *Serratia marcescens* culture filtrates and Shear polysaccharide are antigenic and neutralizable by immune sera. Cross

neutralization experiments demonstrate a close antigenic relationship between the factors present in both materials. It is significant that the chemical treatment, and tryptic digestion in particular, employed by Hartwell, Shear, and Adams (12), failed to reduce or modify to any measurable degree the antigenicity and specific neutralization of their final product.

DISCUSSION

As already pointed out the concentrates of *Serratia marcescens* culture filtrates successfully purified by Shear and his co-workers are endowed with high potency in producing hemorrhage and necrosis in experimental tumors.

By means of "end-point" titrations it was found that the minimal provocative dose was approximately 8 μgm. per kg. of body weight of the rabbit. In the experiments of Shear and his co-workers 0.1 μgm. was the minimal dose of the same polysaccharide necessary to produce hemorrhage in a mouse tumor. If the average weight of a mouse is taken to be 20 gm., then the minimal effective dose per gram of body weight of the mouse and the rabbit are 0.005 μgm. and 0.008 μgm., respectively. Considering that the amounts employed in the "end-point" titrations are extremely minute, the doses seem to be sufficiently near to each other to support the evidence on the close relationship between the tumor-destroying and phenomenon-producing factors of the bacterial substances. Obviously the comparison is being suggested with considerable reservation, since allowance has to be made for possible differences in the reactivity of the mouse tumors and the prepared skin sites of the rabbit, and for the experimental error of *in vivo* quantitative titrations.

TABLE III: SERUM NEUTRALIZATION OF THE PROVOCATIVE FACTORS OF THE PHENOMENON OF LOCAL SKIN REACTIVITY TO *Serratia marcescens*

Provocative injection Mixture ingredients							
Neutralizing antibody			Toxic substances				
				Amount			
Serum designation	Immunizing antigen	Amount	Material	Provoc. units	Conc.	Results	Provoc. units neutralized by 0.75 cc. of serum
I	P2-Shear polysacchar.	0.5 cc.	P2-Shear polysacchar.	1.25	10γ	NN	
I	" "	0.5 cc.	" "	5	40γ	NN	
I	" "	0.75 cc.	" "	2.5	20γ	CN	
I	" "	0.75 cc.	" "	5	40γ	CN	<5
IIb	" "	0.5 cc.	" "	5	40γ	NN	
IIb	" "	0.75 cc.	" "	2.5	20γ	CN	
IIb	" "	0.75 cc.	" "	3.75	30γ	CN	
IIb	" "	0.75 cc.	" "	5	40γ	IN	
IIb	" "	0.75 cc.	" "	10	80γ	NN	4.4
IIb	" "	0.75 cc.	*S. marcescens* cult. filtr.	4	1.6 cc.	CN	<4
V	*S. marcescens* cult. filtr.	0.25 cc.	" "	2½	1 cc.	CN	
V	" "	0.75 cc.	" "	4	1.6 cc.	CN	
V	" "	0.75 cc.	" "	5	2 cc.	CN	
V	" "	0.75 cc.	" "	10	0.2 cc. lyophil. conc.	CN	<10
V	" "	0.75 cc.	P2 Shear concentrate	2.5	20γ	CN	
V	" "	0.75 cc.	" "	5	40γ	CN	<5

Conc. = concentration.

The studies reported in this paper were deemed necessary in order to bring further evidence that the tumor-destroying factors in bacterial filtrates are closely related to or identical with the bacterial substances capable of producing the phenomenon of local tissue reactivity.

Strains of *Serratia marcescens* differ from one another in their ability to produce the active principles of the phenomenon. While some of the strains give inactive filtrates (27), Aitoff, Dion, and Dobkevitch (1) isolated from the sputum of a case of pneumonia a strain of *Serratia marcescens* pathogenic for mice, rats, guinea pigs, and rabbits. The culture filtrates of this strain gave intense hemorrhagic reactions of the phenomenon. In the experiments of this paper the strain used by Shear and Turner (24) for preparation of their material yielded phenomenon-producing principles of moderate potency in filtrates of cultures in a simple synthetic medium.

In the following step of the studies it was attempted to determine whether the active principles of the phenomenon of local skin reactivity undergo any antigenic alteration as a result of the chemical treatment employed for preparation of the concentrates. The studies were considered especially pertinent to the problem, inasmuch as the tryptic digestion used in the process of purification could conceivably produce some changes in the protein moiety of the toxins.

The filtrates and the polysaccharide were found to be highly antigenic since following a course of immunizing injections into rabbits they stimulated production of antisera that gave precipitation reactions

with the filtrates and concentrates and neutralized the active principles. By means of cross precipitation and cross neutralization experiments a close antigenic relationship was shown to exist between both materials. The cross precipitations suggested, however, that the culture filtrates possess a broader and possibly higher antigenicity than the polysaccharide.

In attempting to evaluate the results described above it is of interest to quote some of the most important studies by previous investigators on the trypsin digestion of antigens and toxins. Wells (28) digested bovine serum with trypsin for a period of 16 months. The power of the serum to sensitize guinea pigs decreased with decrease in coagulable protein. The sensitization was possible, however, even after the biuret reaction became negative and the coagulable protein could no longer be detected. According to Obermayer and Pick (17) precipitins may be obtained by immunizing animals with biuret-negative, trypsin-digested, coagulated bovine serum and egg albumen. The antisera may not react with the initial nondigested proteins but only with the digested material. Mayer (16), Rostoski (20), and Sacconaghi (21) found that trypsin fails to impair the specific antigenicity of the agglutinogens and precipitinogens of trypanosomas. Landsteiner and his associates (15) produced acetylated protein preparations that were effective antigens despite complete resistance to trypsin and pepsin. Tryptic digestion of short duration produced no effect upon the activity of ricin (14), abrin (13), and dysentery toxin (5). Prolonged digestion injured the dysentery toxin (9) and ricin (18). In a recent publication Dubos, Hoberman, and Pierce (6) reported the toxin of *Shigella dysenteriae* completely resistant to proteoloytic enzymes. While trypsin easily destroyed snake venoms (8) and completely or partly inactivated diphtheria toxin (4), botulinus toxin was unaffected by peptic and tryptic digestions of short duration. A tryptic digest containing 0.5 per cent sodium carbonate incubated for 4 hours at 38° C. did not weaken botulinus toxin (22).

On the basis of all the evidence available, only a part of which has been quoted here, Pick and Silberstein (19) conclude that the following various effects of trypsin upon antigenic proteins may be observed, namely: (a) alteration of the constitutional specificity without change in species specificity; (b) alteration of species specificity without effect upon the constitutional specificity; (c) alteration of both constitutional and species specificities; and, finally; (d) complete destruction of antigenicity.

Thus in the light of the investigations just quoted, the results of the experiments recorded in this paper may be stated as follows:

The chemical treatment, including tryptic digestion, of *Serratia marcescens* concentrates employed by Shear and his co-workers brings about concentration and purification of the active principles of the phenomenon of local skin reactivity without inducing any measurable alteration in their antigenicity. By means of the same treatment the precipitinogens possibly undergo a certain narrowing of specificity. Further observations by means of cross absorption tests remain necessary on this point.

In contradiction to the conclusions above an assumption could be made that the antigencity may be due to some minute quantities of contaminating material but not to that of the active principles themselves. This assumption is excluded clearly, however, by the fact that the antisera prepared by immunization with the polysaccharide contain potent antibodies neutralizing the active principles of the phenomenon.

The conclusions of these investigations are not intended to imply that a pure polysaccharide is capable of functioning as a complete antigen. The question whether some carrier substance possibly present in minute quantities in the preparation is necessary for production of actively acquired antibodies still remains unanswered in the studies described.

SUMMARY

Filtrates of cultures of *Serratia marcescens* in a simple synthetic medium, and their concentrates prepared by Shear and his co-workers, are capable of eliciting the phenomenon of local skin reactivity.

The phenomenon-producing principles are closely related to or identical with the factors capable of inducing hemorrhages and regressions of mouse tumors.

The chemical treatment, including tryptic digestion, employed by Shear and his co-workers for preparation of the product brings about concentration and purification of the active principles of the phenomenon without inducing any measurable alteration in the antigenic specificity as shown by the immunizing value, the precipitation, and the neutralization reactions of the materials.

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The Cancer Congress of Guadalajara (Mexico)

This, the first cancer congress ever held in the Republic of Mexico, met at the School of Medicine of Guadalajara, Jalisco, from the 31st of October until the 6th of November, 1943. The Congress was sponsored by the President of the Republic, General M. Avila Camacho, the Federal and State Departments of Public Health, and the University of Guadalajara. It was organized by Dr. G. Vazquez Arroyo, President of the Executive Committee, with the active cooperation of Dr. E. Garcia Ruiz, general secretary; Drs. M. Baeza, M. Riebeling, and N. Pallares, secretaries; Dr. S. Hernandez Urtiz, treasurer; and several others acting as secretaries of the various sections. The Congress was held in conjunction with a general medical meeting, that of the "Segunda semana medica de Occidente," where topics of general medicine were discussed. This was a fortunate occurrence, for the scope of the meeting was widened and, in addition, general practitioners and specialists in other fields had the opportunity of getting first hand information about cancer. Moreover, the sessions were always attended by almost the entire student body of the medical school of the University. Thus the Congress fulfilled a didactic mission. Since the full proceedings will be soon published, this report will be limited to a general idea of its activities.

The trend of the meeting was along clinical lines. In the main amphitheatre of the School of Medicine lectures on the most important types of cancer were given daily by authorities on their respective subjects. These lectures were largely of the ex-cathedra variety, which is especially interesting for general practitioners and students. A list of some of them follows: Cancer of the brain by Dr. Clemente Robles, of Mexico, D.F.; Cancer of the uterus, by Dr. M. Garcia; Cancer of the larynx, by Dr. F. E. Le Jeune; Cancer of the lung, by Dr. A. Ochsner (the three latter speakers from Tulane University); Cancer of the skin, by Dr. J. Bejarano, of Mexico, D.F.; Cancer of the breast, by Drs. J. Morones and J. Noyola, of San Luis de Potosi, Mexico; Gynecological cancer, by R. Amor, of Mexico, D.F.; Cancer in urology, by Drs. A. Villanueva and A. Madrid, of Torreon, Mexico.

Other sections of the Congress dealt with subjects of a more specialized sort. The following are cited almost at random: A contribution to giant cell tumors of the lower jaw, by Dr. A. Martinez Calderon, of Coahulla, Mexico; Treatment of mouth cancer, by Dr. German Garcia, of Mexico, D.F.; Notes on uterine cancer, by Drs. J. Velasquez Uriarte and O. Rojas, of Mexico, D.F.; X-rays in the diagnosis of uterine cancer, by Dr. A. Wiesman, of the Metropolitan Hospital of New York. A symposium covering the clinical, histopathological, and hematological aspects of Hodgkin's disease was given by Drs. Chavez, Mendiola, and Amozurrutia respectively. An interesting device (broncho-catheter) to secure biopsies of lung growths was presented by Dr. A. Perez Ara, of Havana, Cuba.

Contributions to the theoretical and experimental sides of the problem were few. Among them should be mentioned a comprehensive review by Dr. F. Giral, of Mexico, D.F., of hydrocarbons, bile acids, and sex hormones in relation to cancer, and an account by Dr. F. Duran-Reynals of the experimental work on cancer carried out in various departments of Yale University.

The social aspects of the disease and plans for cancer control were fully covered by several authorities. Dr. R. R. Spencer, Director of the National Cancer Institute, Bethesda, Maryland, gave a paper read by Dr. Riebeling in Spanish on the program of the cancer service of the United States of America while other papers on comparable subjects, but of a clinical type, were read by Dr. I. Millan, head of the Cancer Unit of the Hospital General of Mexico and Dr. M. Baeza, secretary of the recently inaugurated Cancer Unit of Guadalajara Medical School. Dr. F. Valdes Villarreal, of the Hospital General, read an excellent paper that was accompanied by moving pictures on training in the diagnosis of cancer.

Operative clinical sessions on interesting cases were carried out every day in Civilian Hospital of Guadalajara, connected with the Medical School. Among them one may mention a thyroidectomy by Dr. G. Vazquez Arroyo, a gynecological operation by Dr. R. Amor, and a resection of the lower jaw by Dr. A. Velasco Zimbron.

The Congress brought together a large number of physicians and scientists from different nations, the largest representation being that of the United States, with 40 members. Naturally, the visitors had an ample opportunity of getting acquainted with their colleagues south of the Rio Grande and could appreciate their magnificent skill as surgeons, their extraordinary potentialities as men of science, and their generosity and charm as hosts. One is tempted, indeed, to describe the varied and bountiful style of the entertainments, which were made possible by a generous grant from the Mexican Government and contributions from several commercial firms. Dr. G. Vazquez Arroyo was the central figure of the Congress. A large part of its scientific success was due to his initiative and activity,

while Mrs. Vazquez Arroyo was at the head of the committee that organized the program of entertainment. Many of the foreign delegates visited the scientific institutions in other cities of the Republic, notably Mexico City. They thus had the chance of watching a crucial stage in the scientific development of a nation whose racial sources are so different from those of the United States. The visitors learned how extremely brilliant Mexican science is as far as individuals are concerned, but how deficient in several ways in respect to the organization of groups. However, signs of the creation of a proper environment are everywhere, and centers of learning such as the still unfinished Institute of Cardiology, directed by Dr. I. Chavez; the Institute of Public Health and Tropical Diseases; and still others, are as good as any similar institutions in the United States. The influence of scholars, refugees from the Spanish Republic and the best minds in their various lines, begins to be felt, and men like Blas Cabrera, the Girals, Bolivar, Costero, Carrasco Formiguera, Folch, German Garcia, and many others, are doing their utmost to contribute to progress. It is easy indeed to foresee a brilliant future for Mexican science.

FRANCISCO DURAN-REYNALS

Abstracts

Experimental Research, Animal Tumors

Aqueous Soap Solutions of Carcinogenic Hydrocarbons. Beck, S. [Glasgow Royal Cancer Hosp., Glasgow, Scotland] *Nature, London,* **152**:537. 1943.

An ether solution of a hydrocarbon (*e.g.,* 20-methylcholanthrene or 3,4-benzpyrene) is added to an aqueous solution of ether soap ("1 cc. of the ethereal soap solution used contains 1.8 gm. soap"), diluted with water to obtain the desired percentage of hydrocarbon and of soap, and the ether removed by boiling. "Fluorescence spectroscopy reveals identical bands for 3:4-benzpyrene in aqueous soap solution and in acetone." The solution becomes opalescent after a few hours, but "even so the particles are much smaller than those of colloidal suspensions." "Rats show no aversion to drinking 0.01 per cent hydrocarbons in 7.5 per cent aqueous soap solution, and have showed no toxic effects after drinking it daily for six weeks."—I. H.

Mammary Carcinoma in Mice Following the Intranasal Administration of Methylcholanthrene. Orr, J. W. [Univ. of Leeds, England] *J. Path. & Bact.,* **55**:483-488. 1943.

Strains IF and CBA are completely free from spontaneous mammary cancer. Females of both strains treated intranasally with an oily solution of methylcholanthrene developed mammary cancer in about two-thirds of the survivors. Males of the IF strain that had been feminized by an estrogen (triphenylethylene) also developed breast cancer (11 of 16 mice) after methylcholanthrene given intranasally. This second experiment disposes of the objection that the females in the first might have transferred methylcholanthrene to the mammary ducts while cleaning themselves. No mammary ducts were found in estrogen-treated males.

Numerous skin tumors were found. "Within each strain there is no evidence that the frequency of skin tumours is significantly different in mice which developed mammary tumours."—I. H.

Intermediate Stages in the Metabolic Conversion of Benzpyrene to 8-Hydroxy-benzpyrene in Mice. Weigert, F., and Mottram, J. C. [Mount Vernon Hosp., Northwood, Middlesex, England] *Biochem. J.,* **37**:497-501. 1943.

Benzpyrene after it is injected into mice appears in the digestive tract as 2 derivatives BPX (blue fluorescence) and BPF (blue-green fluorescence, probably 8-hydroxybenzpyrene). BPX is found in the small intestine and in bile and can be adsorbed on Al_2O_3; both adsorbate and eluate have the same (blue) fluorescence spectrum, which is different from the spectrum of BPX before adsorption. BPF is found in the large intestine; its adsorbate on Al_2O_3 fluoresces green, but the eluate fluoresces whitish blue; the fluorescence spectra of the adsorbate and of the eluate differ from that of BPF before adsorption. BPX is changed into BPF after it has passed through the ileocecal valve, or by heating at 100–200° C., or more slowly by standing (prevented by addition of formalin). "Slight differences in the rate at which the green zone moved down the chromatographic column were seen when (BPF) was prepared from different sources. Hence (BPF) probably comprises a group of complexes which contain various cell constituents in combination with 8-hydroxybenzpyrene." [(BPF) signifies the derivative formed by adsorbing BPF on Al_2O_3.] A red fluorescing zone (not due to benzpyrene-5,8-quinone) accompanied particularly strong green-fluorescing adsorbates. The authors conclude that "It has been shown by fluorescence chromatography of quite fresh extracts from organs of mice which had been treated with benzpyrene that the hydrocarbon is not metabolized directly to 8-hydroxybenzpyrene but that the transformation passes through three intermediate stages which have been studied separately."—I. H.

Factors That Alter the Fluorescence of Certain Carcinogens. Weil-Malherbe, H. [Royal Victoria Infirmary, Newcastle upon Tyne, England] *Cancer Research,* **4**: 102-105. 1944.

Observations are reported and discussed with reference to the experiments of Miller and Baumann. The following points are made:

The chemical classification of a solvent has no relation to the fluorescence intensity of the hydrocarbons dissolved in it.

For the particular case of 3,4-benzpyrene solution in ethanol-water mixtures it can be shown that the differences in fluorescence intensity according to the percentage of solvent composition are entirely due to a quenching effect of dissolved O_2. The differences disappear in N_2. In many other cases the solvent effect on fluorescence intensity can be largely accounted for by the quenching effect of O_2, though there are exceptions where a solvent effect proper persists even in the absence of O_2.

The stability of the fluorescence of benzpyrene solutions also depends on the solvent. No appreciable change occurs in hexane or benzene during a 20 minute irradiation. In acetic acid, and especially in 80% acetic acid—20% water mixture, photo-oxidation takes place in the presence of O_2. In ethanol and in mixtures of 60% ethanol with 40% *N*/10 HCl, water or *N*/10 NaOH a fairly slow nonoxidative fall of fluorescence, increasing in the order named, is observed.

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

The rapid destruction of fluorescence in chloroform is confirmed and is shown to be a nonoxidative reaction.

Inhibitors of fluorescence in solution belong to one of three categories: (a) substances causing a *reversible* photochemical reaction ("genuine quenching"); (b) substances causing *irreversible* changes of the fluorescent material; (c) substances that absorb the exciting wave lengths ("filter effect"). Many solvents in which hydrocarbons do not fluoresce belong to this class.

An unsaponifiable fraction of mouse tissues is less susceptible to the quenching effect of O_2 than carcinogenic hydrocarbons. It is recommended that fluorimetric determinations of hydrocarbons be carried out in N_2.—Author's summary.

Multiple Mesenteric Sarcomata in Rats Following Ingestion of Heated Lard. Peacock, P. R., and Beck, S. [The Glasgow Royal Cancer Hosp., Glasgow, Scotland] *Brit. J. Exper. Path.*, **24**:143-146. 1943.

This paper deals with mesenteric tumors that occurred in the course of experiments designed to test Roffo's report that fats become carcinogenic when heated at 350° C. The experimental animals were male Norwegian hooded rats fed on an adequate diet (N rats) and also male Wistar rats fed on a restricted diet of white bread and milk (W rats). The heated lard consumed averaged 6 gm. weekly per rat.

Fifty N and 20 W rats were fed lard heated for 1 hour at 350° C. Two N and 1 W rat developed multiple mesenteric sarcoma; 3 other N and 2 other W rats were found to be infested with Cysticercus fasciolaris (1 of the W rats had liver sarcoma due to the worm). Four W rats had multiple ulcerated papillomas in the forestomach.

Thirty-five N and 20 W rats received lard heated for 12 hours at 220° C. One N rat had mesenteric sarcomas; 2 W rats (1 with liver sarcoma due to the worm) were infested; 2 W rats had stomach papillomas.

Fifty N rats received lard heated for 4 hours at 220° C. Two were infested, one of which had a mesenteric sarcoma very close to the intestinal site of the worm.

Twenty-four controls (16 N and 8 W rats) had 6 gm. of unheated lard per rat weekly. One N and 1 W rat were found infested with the worm.

It is concluded that the evidence points to the presence in fats heated to about 350° C. of some factor capable of inducing malignant mesoblastic tumors along the path of their absorption in addition to the benign gastric lesions previously described.—I. H.

Heated Cholesterol as a Potential Carcinogen. Kirby, A. H. M. Proceedings of the Biochemical Society. *Biochem. J.*, **37**:xi. 1943.

(1) Cholesterol, (2) cholesterol heated at 270-300° (unchanged sterol removed), (3) residue from (2) after removal of cholesterone and dicholesteryl ether, (4) Δ3,5-cholestadiene were added separately to the diets of groups of rats for 24 months. Only one papilloma and hyperkeratosis (of forestomach ?) was observed. (5) Heated cholesterol esters, (6) cholesterol heated at 430°, also given by mouth, gave no tumors in 15 months. Oily solutions of the products listed above, injected subcutaneously into 90 rats, gave 2 fibrosarcomas (which products were effective is not stated). Examination of absorption spectra gave no support for Roffo's claim that heated cholesterol contains a polycyclic hydrocarbon very similar to 3,4-benzpyrene.—I. H.

The Effect of Glycocyamine on the Formation of Tumours Induced by Acetyl-amino-fluorene. Bielschowsky, F. Proceedings of the Biochemical Society. *Biochem. J.*, **37**:xv. 1943.

The addition of 2-acetyl-amino-fluorene to the diet of rats produces tumors in different organs. In female rats the majority of the tumors are mammary adenocarcinomas. Glycocyamine added to the acetyl-amino-fluorene containing diet accelerates the appearance of mammary tumors and also increases the number of liver tumors in the females. "These results suggest that the reduction of available labile methyl-groups brought about by the conversion of glycocyamine into creatine enhances the carcinogenic action of 2-acetyl-amino-fluorene."—I. H.

Attempts to Induce Stomach Tumors. II. The Action of Carcinogenic Hydrocarbons on Stock Mice. Peacock, P. R., and Kirby, A. H. M. [Glasgow Royal Cancer Hosp., Glasgow, Scotland] *Cancer Research*, **4**:88-93. 1944.

Stock mice receiving an adequate diet with the addition of either 3,4-benzpyrene or 20-methylcholanthrene in solution in olive oil or cod liver oil developed papillomas of the forestomach with a tendency to malignant evolution. Even a small number of positive results in the experimental induction of tumors of the forestomach of mice must be considered significant in view of the extreme rarity of such tumors in this species.

Unlike the diffuse benign gastropapillomatosis associated with dietary deficiencies, papillomas induced with carcinogenic hydrocarbons (a) tend to be localized and invasive, and (b) are not prevented or influenced by the addition to the diet of large amounts of vitamin A.

The relative values of stock mice and inbred lines for investigating the competence of tissues to react to carcinogens are discussed.—Authors' summary.

Attempts to Induce Stomach Tumors. III. The Effects of (a) A Residue of Cholesterol Heated to 300° C., and (b) Δ3,5-Cholestadiene. Kirby, A. H. M. [Glasgow Royal Cancer Hosp., Glasgow, Scotland] *Cancer Research*, **4**:94-97. 1944.

Rats on an adequate basal diet, fed the residue (left after the removal of dicholesteryl ether and Δ4-cholestenone) from cholesterol heated to 300° C., at a level of 20 mgm. daily for 2 years, showed no tumor of the forestomach nor of the glandular zone.

Other rats fed Δ3,5-cholestadiene at a level of 25 mgm. daily for 2 years plus an adequate basal diet, showed no tumor in either part of the stomach. It seems unlikely that this diene is concerned in the avitaminosis A induced in rats by feeding heated fats.

A large, inorganic bladder stone is reported in one rat of this series.—Author's summary.

Studies in Esterase (Butyric) Activity. III. The Effect of Foster Nursing on the Esterase Content of Blood Serum and Liver of Strains of Mice Susceptible or Insusceptible to Mammary Cancer.

KHANOLKAR, V. R., and CHITRE, R. G. [Tata Memorial Hosp., Bombay, India] *Cancer Research,* **4**:128-133. 19144.

The effect of foster nursing on the esterase activity of serum and livers of mice, of a high mammary cancer strain (C3H) and a low mammary cancer strain (C57) was studied.

Reciprocal foster nursing resulted in a change in the esterase content of the serum of these mice. The esterase activity in the high mammary cancer strain diminished on foster nursing by the low mammary cancer strain mothers, while the esterase activity in the low mammary cancer strain increased on foster nursing by high mammary cancer strain mothers. These results were statistically significant.

No significant change was observed in the liver esterase on foster nursing.

A probable interpretation of these findings in relation to spontaneous mammary cancer in mice is discussed.—Authors' summary.

Metabolic Studies in Patients with Cancer of the Gastrointestinal Tract. VI. Disorders in Alimentary Digestion and Absorption in Patients Who Have Undergone Total Gastrectomy for Carcinoma of the Stomach. REKERS, P. E., PACK, G. T., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Surgery,* **14**:197-215. 1943.

Three patients who had undergone total gastrectomy for carcinoma of the stomach exhibited a conspicuous steatorrhea, and one of them showed conspicuous creatorrhea that was related not only to the dietary protein but also to the dietary fat since it was increased with an increased intake of fat. The steatorrhea in these 3 patients was related to the dietary fat, possibly related to the protein content of the diet, and probably related to fat-splitting enzymes located in the pancreas. It was not due to a lack of gastric chyme, an intrinsic factor in the gastric juice, insufficient reservoir to hold the ingested food, nor increased intestinal motility. Pancreatic enzyme and beef supplements reduced the steatorrhea.

Anemias of normocytic, normochromic type were noted in these 3 patients. The anemias apparently were not related to deficiency of dietary protein, intrinsic or extrinsic factors, or to the presence of infection. They may have been associated with a disturbance in alimentary digestion and in one case with bone marrow dysfunction.—W. A. B.

Metabolic Studies in Patients with Cancer of the Gastro-Intestinal Tract. VII.—The Influence of Gastric Surgery upon the Chemical Composition of the Liver. ARIEL, I., PACK, G. T., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Ann. Surg.,* **116**:924-927. 1942.

Chemical determinations of the liver were made on 13 patients who had various diseases of the stomach and were subjected to operation. There were 8 cases of carcinoma, and 1 case each of polyp, gastric ulcer, duodenal ulcer, gastritis, and Boeck's sarcoid. Biopsy of the liver was done immediately upon opening the peritoneal cavity and again just before closure. During the course of subtotal gastrectomy there was an average increase of 1% hepatic water, a shift of fluids between intracellular and extracellular positions, an average loss of 45% of the hepatic glycogen, an average increase of 1.97 gm.% of total lipids, and a fluctuation in the content of hepatic protein with an increase in hepatic albumin and a decrease in hepatic globulin.—W. A. B.

The Effect of Exercise on the Growth of a Mouse Tumor. RUSCH, H. P., and KLINE, B. E. [Univ. of Wisconsin, Med. Sch., Madison, Wis.] *Cancer Research,* **4**:116-118. 1944.

Albino mice were placed in a motor-driven rotating cage and subjected to certain periods of forced exercise preceding and following inoculation with a transplantable fibrosarcoma. The rate of growth of the tumors was then compared to a control series in mice receiving the same daily caloric equivalent of food but not subjected to forced exercise. The exercised mice gained less weight and the rate of tumor growth was also less than that observed in the control series.—Authors' abstract.

Lymphoid Tumors in Mice Receiving Steroid Hormones. GARDNER, W. U., DOUGHERTY, T. F., and WILLIAMS, W. L. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **4**:73-87. 1944.

Studies were undertaken on 822 control mice, 1,799 estrogen-treated mice, 378 estrogen-androgen-treated mice, and 65 androgen-treated mice from 7 inbred strains. Humoral treatment was begun when the animals were 22 to 141 days old and continued for either 10 weeks to 3 months or until death. All hormones were injected subcutaneously in oily solutions or implanted subcutaneously as pellets. Nine estrogenic chemicals at one or several dosage levels were used.

Malignant lymphomas occurred in 1.34% of the control mice, 11.9% of the estrogen-treated, 2.34% of the estrogen-androgen-treated, and in 1.54% of the androgen-treated mice. The incidence was approximately 15% among the estrogen-treated mice of 3 strains (C3H, CBA, and PMO), 5% in the JK and C12I, and 2% in the C57 and A. The incidence of spontaneous lymphomas was not paralleled by the incidence of such tumors among the treated mice. The treatment of mice with large doses of estrogen for periods of 10 weeks to 2 months was followed by an incidence of lymphomas as high as, or higher than, that occurring after continuous treatment. Small doses of hormone were associated with a low incidence of lymphomas. Female mice treated with estrogens tended to have a higher incidence of tumors than males. The simultaneous administration of androgen tended to prevent the high incidence of lymphomas. The different estrogenic chemicals, when given in physiologically comparable amounts, were approximately equally effective.

The tendency for estrogens to be more effective in increasing the incidence of lymphoid tumors among some strains of mice than among others could not be associated with any concomitant peculiarities of these strains in their response to estrogen, such as the tendency to acquire mammary, hypophyseal, or testicular tumors or the responsiveness of the skeletal tissues.—Authors' abstract.

Production of Malignancy in Vitro. I. Method of Cleaning Glassware. EARLE, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:131-133. 1943.

For thorough cleaning of glassware used in *in vitro* methylcholanthrene carcinogenesis experiments, detergents such as soap or trisodium phosphate are considered inade-

quate, while dichromate-sulfuric acid cleaning solution is objectionable because of adsorption of chromium salts on the glass. The best results are obtained with hot 80% sulfuric acid plus a small amount of nitric acid. Tests show that 1 mgm. lots of crystalline methylcholanthrene alone or with serum added are charred within a few minutes at room temperature by this solution. With gentle warming the charred material is cleared and put into solution.

The glassware cleaning apparatus is described in detail.—R. B.

Production of Malignancy in Vitro. II. Photomicrographic Equipment. EARLE, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:135-145. 1943.

The photomicrographic apparatus is constructed in such a manner that all manipulations can be carried out while the image is under observation. Since the microscope is inverted and enclosed in an air bath, photographs can be taken of living cultures in their normal position in Carrel flasks or hollow ground slides. The whole apparatus is mounted on a concrete base which in turn is insulated from the floor by Vibracork. Details of the construction of the apparatus and photographs illustrating it are given in the paper.—R. B.

Production of Malignancy in Vitro. III. Microcinematographic Equipment. EARLE, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:147-164. 1943.

The equipment is designed for taking motion pictures simultaneously of fibroblast cultures exposed to methylcholanthrene and of control cultures.

Many interesting special features are incorporated into the apparatus to make it as convenient and automatic as possible.—R. B.

Production of Malignancy in Vitro. IV. The Mouse Fibroblast Cultures and Changes Seen in the Living Cells. EARLE, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:165-212. 1943.

Fibroblasts from a 100 day old C3H male mouse were cultured for 291 days on a medium consisting of chicken plasma, horse serum, chick embryo extract, and saline. Experimental cultures were then exposed to 1 γ of 20-methylcholanthrene per cc. of the fluid phase of the medium for periods ranging from 6 to 406 days, after which the carcinogen was omitted from the medium and the cultures observed for an additional 257 to 536 days.

The growth of carcinogen-treated fibroblasts, as determined from measurements of diameters of cultures, was retarded beginning at 6 to 19 days. Morphologic changes were apparent after the cultures had been exposed for 40 days to the carcinogen. These changes consisted of a shortening of cell processes followed later by the development of lateral irregularities on the blunted processes. These irregularities were suggestive of amoeboid rippling or frothing. With continued exposure to the carcinogen the cells developed numerous short lateral processes and began to adhere to one another forming strands or ribbons of cells, and finally massive sheets of cells. The cytoplasm of cells treated by the carcinogen for long periods became very granular.

These morphologic changes occurred gradually and became more pronounced as the length of the period of exposure to the carcinogen increased. Growth rate also was progressively retarded. After 406 days in the methylcholanthrene-containing medium, cultures grew very slowly and would probably have died with additional exposure to the carcinogen. After removal from the carcinogen the cultures grew somewhat faster, but still at a reduced rate compared with the controls. This apparently permanent reduction in growth rate was proportional to the length of time during which the cultures had previously been exposed to the carcinogen. Observations extending for more than 1 year following removal of the carcinogen from the culture medium showed that the morphologic changes also persisted unchanged.

About 21 months after the original explantation, the control cultures began to display alterations of the same type as described above for the carcinogen-treated cultures. These changes in the controls occurred 2 to 8 months later than they did in the experimental cultures and were much less extensive on the whole. They could have occurred spontaneously, but the author inclines to interpret them as resulting from unavoidable trace contamination of the control cultures with methylcholanthrene.

The paper contains a detailed description of the tissue culture technic followed in these experiments.—R. B.

Production of Malignancy in Vitro. V. Results of Injections of Cultures into Mice. EARLE, W. R., and NETTLESHIP, A. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:213-227. 1943.

Cultures of C3H mouse fibroblasts exposed to methylcholanthrene as described in the preceding abstract were inoculated, usually intramuscularly, into young C3H mice. Control cultures were similarly inoculated. Sarcomas developed from all strains of fibroblasts, both carcinogen-treated and controls, after latent periods of 9 to 14 days or longer. These sarcomas have been carried on by subinoculation into C3H mice for 14 tumor generations. Metastases occurred in 6 out of 77 mice inoculated with 2 of the carcinogen-treated fibroblast strains. The remaining 3 carcinogen-treated strains and the control cultures did not produce metastases.

The experimental fibroblast strains tested for malignancy had been exposed to methylcholanthrene in culture for periods ranging from 6 to 406 days and then cultured for varying periods in the absence of the carcinogen. Seventy-six per cent of the inoculations of cultures treated with the carcinogen for 6 days produced tumors. Longer periods of exposure produced increasingly severe morphologic alterations in the cultures and a decrease in the percentage of tumors in inoculated mice. Cultures exposed to the carcinogen for 32, 111, 184, and 406 days produced tumors in 20%, 46%, 7.6%, and 7.6% of the inoculated mice respectively.

The percentage of inoculations giving rise to tumors was small (7.6%) for one control fibroblast strain displaying very slight morphologic alteration, but high (86%) for the other control strain which displayed more definite alterations.—R. B.

Production of Malignancy in Vitro. VI. Pathology of Tumors Produced. NETTELSHIP, A., and EARLE, W. R. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:229-248. 1943.

Autopsies were performed on a total of 838 C3H mice

carrying inoculations or subinoculations of (a) C3H fibroblast cultures exposed to 20-methylcholanthrene, and (b) untreated control cultures. Tumors were found in 578 mice. Histological studies on 285 of these tumors showed that they were sarcomas made up usually of large spindle cells. The tumors invaded a large variety of organs and tissues. In 5 cases there were definite metastases to the lungs and in 1 case lungs and mediastinum were involved probably by metastases, but possibly by direct extension from a heavily invaded peritoneum.

The various fibroblast strains produced sarcomas with different characteristics as follows: Control cultures and cultures exposed to the carcinogen for 6 or 32 days produced tumors with quite uniform cell pattern and limited capacity of invasion. There were no metastases and no appreciable numbers of giant cells or abnormal mitoses. Cultures exposed 111 days to the carcinogen gave rise to tumors with increased variability of cell pattern, and some giant cells, and abnormal mitoses. These tumors were more invasive and produced metastases in 5 cases. Finally, the tumors arising from cultures exposed 184 and 406 days to the carcinogen displayed great variability of cell pattern, numerous giant cells, and abnormal mitoses. The tumors were invasive but metastasized in only 1 case.—R. B.

Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture. II. The Delayed Lethal Effect. Doljanski, L., Goldhaber, G., and Halberstaedter, L. [Hebrew Univ., Jerusalem, Palestine] *Cancer Research,* **4**:106-109. 1944.

The delayed lethal dose of x-rays for rat sarcoma cells cultivated *in vitro* is 2,000 r. This is less than half the equivalent dose for chicken normal fibroblasts, of which the proliferative capacity is irreversibly checked only after irradiation with 5,000 r.—Authors' summary.

Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture. III. Further Studies on the Inhibitory Effect of X-Rays on Cell Outgrowth. Goldhaber, G., Doljanski, L., and Halberstaedter, L. [Hebrew Univ., Jerusalem, Palestine] *Cancer Research,* **4**:110-112. 1944.

The minimum dose of x-rays that causes appreciable inhibition of growth rate in an irradiated sarcoma cell culture is 500 r. The corresponding dose for normal chicken fibroblasts is 2,500 r.—Authors' summary.

The Retention of Radioactive Phosphorus When Administered in Different Forms. Warren, S., and Cowing, R. F. [New England Deaconess Hosp., Boston, Mass.] *Cancer Research,* **4**:113-115. 1944.

In the course of determining the value of radioactive phosphorus as a therapeutic agent in leukemia, several chemical forms of the substance have been used. Small amounts of radioactive magnesium ammonium phosphate, phosphoric acid, or disodium phosphate may be used practically interchangeably so far as therapeutic effect and rate of excretion are concerned. In the preparation of radioactive phosphorus for therapeutic administration to human patients, phosphoric acid or magnesium ammonium phosphate are more readily prepared than dibasic sodium phosphate.—Authors' abstract.

H 11 for Cancer. Barnett, J. W. Correspondence. *Brit. M. J.,* **2**:589-590. 1943.

The author has had "many failures and a few striking results" and records in detail the successful treatment of a case of very extensive recurrence after excision of both breasts for carcinoma.—E. L. K.

The Role of Humoral Antagonism in Heteroplastic Transplantation in Mammals. Harris, M. [Univ. of Penn., and Wistar Inst., Philadelphia, Pa.] *J. Exper. Zool.,* **93**: 131-145. 1943.

Adult mammals show a natural resistance to alien transplants, whether the graft consists of normal or tumor tissues. Tissue culture methods were used to study the nature of the humoral factors or "cytotoxins" responsible for this resistance. The technic consisted in injecting King A rats with tumor BA_1 from mice of the Bagg albino strain, and then testing the effect of serum from the rats on cultures of mouse and other cells. The results showed that the serum so derived was toxic to all mouse cells, tumor or normal, regardless of the strain from which the cells were taken (Andervont, Bagg albino, or C57 black). However, the serum was not toxic to rat sarcoma, heart, or spleen, cultured in the same roller tubes as the mouse tissues for direct comparison. Thus the inoculation of mouse sarcoma cells into rats produced not an anti-tumor serum, but rather a general "anti-mouse" serum, the reaction being presumably of an antigen antibody nature.—J. B. B.

Growth of a Dibenzanthracene-Produced Mouse Sarcoma in the Chorio-Allantoic Membrane of the Chick. Jacoby, F., McDonald, S., and Woodhouse, D. L. [Med. Sch., Univ. of Birmingham, England] *J. Path. & Bact.,* **55**:409-417. 1943.

Tumors grew in 18% of surviving chick embryos, but further passage into mice was successful only so far as the second generation. The original paper should be consulted for a detailed description of the histological features of this tumor.—I. H.

Experimental Brain Tumors. IV. The Incidence in Different Strains of Mice. Zimmerman, H. M., and Arnold, H. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **4**:98-101. 1944.

These experiments were devised to test the validity of the hypothesis that genetic constitution is an important factor in the incidence of primary intracranial neoplasms induced with pure methylcholanthrene implanted intracerebrally. Of 6 strains of mice tested, namely, C3H, ABC albino, Bagg albino, C57 black, A albino, and dba, the first 4 strains yielded an incidence of 50% or better in primary intracranial neoplasms, whereas the last 2 strains were poor with respect to carcinogen-induced brain tumors. Only 4 of 22 A albino mice and 2 of 19 dba mice developed intracranial neoplasms. The incidence of brain tumors induced with methylcholanthrene is in no way related to the propensities that the different mouse strains show in regard to the development of spontaneous mammary tumors. Both the C3H and dba strains, for example, have a high incidence of spontaneous tumors of this variety, whereas the C57 black strain yields extremely few such tumors. Of 29 inbred dba mice (from brother-to-sister matings) only 1 developed an intracranial tumor following the intracerebral implantation of methylcholanthrene. Hybrids obtained by crossing pen-bred C3H and pen-bred dba mice yielded an intracranial tumor incidence

of 26% (25 tumor mice among 96 animals).—Authors' abstract.

The Chemical Aspects of Cancer. Rhoads, C. P. [Memorial Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**: 1-10. 1942.

From experimental work carried out in the author's and other laboratories, the following conclusions can be drawn: dibenzanthracene can be oxidized in the body thus losing its carcinogenic power; the carcinogenic effect of another chemical, butter yellow, can be postponed or prevented by dietary constituents, one of which appears to be riboflavin, and another an unidentified substance present in casein; a continued deficiency of Coenzyme I may be induced in the affected liver cell by the feeding of a deficient diet plus butter yellow; a metabolic derivative of butter yellow that poisons the Coenzyme I system has been isolated, and its toxic effect on normal cells has been demonstrated both *in vitro* and *in vivo;* under certain conditions the cancer cell is immune to the toxic derivative that damages the normal cell. This development of resistance toward a toxic agent may provide an explanation of the mechanism of malignant transformation. A search for a compound that will reverse the phenomenon last mentioned, *i.e.*, damage cancer cells and leave normal cells unharmed, is suggested as the next step in the study.—S. A. G.

The Chemistry of Carcinogenesis and Tumor Growth. Fifth Frank Billings Lecture of the Thomas Lewis Gilmer Foundation, May 28, 1943. Voegtlin, C. [Bethesda, Md.] *Proc. Inst. Med. Chicago,* **14**: 454-467. 1943.

From the biochemical and functional viewpoint, the author suggests the following tentative picture of the cancer process: chemical carcinogenic agents acting in optimum concentration upon genetically susceptible cells set in motion long-continued cell proliferation. This does not necessarily give rise to cancer cells and under certain conditions may be reversible. The cause of malignant change is still a mystery, but it may involve alterations in intracellular enzyme patterns as revealed by comparative studies on malignant tumors and their normal homologous tissues. Progress will depend on a better understanding of the chemistry and physiology of the normal cell.—M. E. H.

Clinical and Pathological Reports

Skin and Subcutaneous Tissues

Treatment of Epithelioma of the Skin of the Ear. Driver, J. R., and Cole, H. N. [Sch. of Med., Western Reserve Univ. and Univ. Hosps., Cleveland, Ohio] *Am. J. Roentgenol.,* **48**:66-73. 1942.

Unusually difficult complications may arise in the treatment of epithelioma of the ear, usually as a result of insufficient or faulty initial treatment. These tumors are no more difficult to treat than other skin tumors if an early diagnosis is followed by proper therapy.

The author treated 130 cases and followed 107 for 1 year or more. Thirteen patients are known to have died of cancer leaving 94 or 87.9% who were possibly cured. The average age of the patients was 64 years, and men outnumbered women in a ratio of 3:1. Sixty-six per cent of the growths were on the helix or posterior auricular fold. The tragus was involved primarily or secondarily in 14 cases, the anthelix in 5, the lobe in 11, and the concha in 4. The primary tumor was in the auditory canal in only one instance, but extension into the canal was found 6 times. The perichondrium was involved in 35 cases (27%). Biopsies of 71 tumors showed 41 (57.7%) squamous cell carcinomas, 24 (33.8%) basal cell carcinomas, 2 basosquamous epitheliomas, 1 cylindroma, 2 melanoepitheliomas, and 1 sarcoma of Kaposi. Radiation therapy was used in most of the cases, although early lesions were treated by electrodesiccation.—C. E. D.

Treatment of Cancer of the Skin of the Nose. Robinson, G. A., and Harris, J. H. [New York, N. Y.] *Am. J. Roentgenol.,* **48**:59-65. 1942.

The nose is the most frequent location of cancer of the skin of the face. Most of the tumors are either of the reticulated or adenoid basal cell type. Small superficial lesions may be treated by monopolar electrodesiccation; more extensive infiltrating growths require x-ray or radium treatment; surgery is necessary in occasional cases. The technique of radiation therapy is discussed at some length with particular stress on the use of adequate dosage. Reconstructive surgery may be necessary after treatment of large lesions. The end results were satisfactory in all but 12 of 146 patients treated by the authors.—C. E. D.

Cutaneous Carcinoma. IV. Analysis of 20 Cases in Negroes. Schrek, R. [Veterans Administration, Hines, Ill.] *Cancer Research,* **4**:119-127. 1944.

The records of Edward Hines Jr. Hospital and the data presented in the literature were analyzed statistically to determine the incidence and the etiologic factors of carcinoma of the exposed and covered skin in the colored race. It is concluded that scars and chronic inflammatory lesions are important etiologic agents in cutaneous cancer of the negro.—Author's abstract.

Nervous System

Lymphoblastoma of the Spinal Cord Simulating Other Organic Diseases: Report of Two Cases. Baker, G. S. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.,* **17**:588-592. 1942.

In the 2 cases reported an unsual type of tumor of the spinal cord was found. Both are particularly interesting since the symptoms had suggested a serious organic disease in a region remote from the central nervous system. In the first case the lesion was operable, and the patient received definite benefit from the removal of the tumor and postoperative roentgen therapy. In the second case the tumor was advanced and biopsy only could be performed. The outlook for return of function in this patient was poor.—J. L. M.

Cysts of the Cerebellopontine Angle Simulating and Masking Acoustic Neurinoma: Report of Two Cases. Svien, H. J., and Love, J. G. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.,* **17**:385-390. 1942.

The authors report a case of chronic cystic arachnoiditis in which symptoms and findings closely simulated those of acoustic neurinoma. A second case is reported in which

a cystic cap (containing xanthochromic fluid) of such magnitude that it was thought to be sufficient to account for the patient's symptoms, overlay a neurinoma of the acoustic nerve.—J. L. M.

Eye

Pathological Findings in Eyes Enucleated for Glaucoma Due to Sarcoma of the Uvea. Bothman, L. [Univ. of Chicago, Chicago, Ill.] *Illinois M. J.*, **83**:173-175. 1943.

Report of 4 cases. No detension operation to save a blind, painful eye should be done in any case in which the etiology of the secondary glaucoma is not definitely known. Such operations may postpone the enucleation of an eye containing a sarcoma.—M. E. H.

Precancerous Melanosis and the Resulting Malignant Melanoma (Cancerous Melanosis) of Conjunctiva and Skin of Lids. Reese, A. B. [Inst. of Ophth., New York, N. Y.] *Arch. Ophth.*, **29**:737-746. 1943.

Malignant melanoma arising from diffuse differentiation acquired in middle age is different from that arising from a nevus. "Cancerous melanosis" seems to be a suitable term for the type of malignant melanoma that arises from acquired precancerous melanosis. There is a general discussion of the appearance and histology of this type of tumor.—E. C. R.

Female Genital Tract

A Review of Ninety-Four Mixed Mesodermal Tumors of the Uterus. With Report of an Additional Case. Glass, M., and Goldsmith, J. W. [Kings County Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **41**: 309-317. 1941.

In the additional case, fibroids as well as a mixed tumor were found. Muscle tissue could not be demonstrated within this mixed tumor. Radical surgery followed by deep x-ray therapy is the treatment recommended.—A. K.

Two Types of Urological Complications Occurring in Patients Treated for Cancer of the Cervix. Hayllar, B. L. [Pennsylvania Hosp., Philadelphia, Pa.] *Urol. & Cutan. Rev.*, **46**:617-619. 1942.

The two types of complications referred to are ureteral stricture and bladder ulceration. Illustrative cases are reported. With proper urological attention the 5 year cure rate may be increased, especially since many complications are not due to the presence of an active tumor.—V. F. M.

Unusual Coexistence of Granulosa Cell Tumor and Ovarian Teratoma Containing Thyroid Tissue. Murray, N. A., Dockerty, M. B., and Pemberton, J. deJ. [Mayo Clinic, Rochester, Minn.] *Am. J. Obst. & Gynec.*, **44**: 134-137. 1942.

A case report. In a woman past the menopause the unusual coexistence of a granulosa cell tumor and an ovarian dermoid cyst containing hyperplastic thyroid tissue was observed.—A. K.

Carcinoma of Bartholin's Gland. Pund, E. R., and Cole, W. C. [Univ. of Georgia Sch. of Med., Augusta, Ga.] *Am. J. Obst. & Gynec.*, **43**:887-890. 1942.

A case of carcinoma of Bartholin's gland is reported, the thirty-ninth to appear in the literature. The tumor was of low malignancy and no metastases were found. The lesion bore a striking resemblance to that of lymphogranuloma venereum. In the vulva the "elephantiasis" was unilateral and confined to the labium majus. The Frei test was repeatedly negative.—A. K.

Cancer of the Cervix as Seen in a Hospital for Far Advanced Cases. Saltzstein, H. C., and Rao, J. [Mercy Hall Tumor Clinic and Hosp., Detroit, Mich.] *J. Mt. Sinai Hosp.*, **10**:156-166. 1943.

In a review of 139 cases of carcinoma of the cervix, the authors conclude that 50% occur before the menopause, the initial symptoms being hemorrhage and leucorrhea. Hysterectomy has practically been eliminated as a form of therapy; x-ray and radium in combination is now the only treatment these far advanced patients receive. Ten per cent of cases still seek their first medical consultation after they have had symptoms more than 1 year. The preponderant symptoms of the far advanced stage are pain and weakness. Ureteral strictures, present in 50 to 75% of these patients at time of death, have been relieved by cutaneous ureterostomies. Rectovaginal and vesicovaginal fistulae are frequent. Uremia starts insidiously and is frequently the cause of death, with sepsis often added. General metastases are more frequent than they were formerly, since many patients now live longer following treatment. In the series of cases reviewed in the present report, no untreated patient lived longer than 3 years. Of 15 treated patients, 10 lived more than 5 years, thus treatment definitely prolonged the lives of many of those who ultimately died.—A. Cnl.

The Present Status in the Treatment of Cervix Cancer. Taussig, F. J. [St. Louis, Mo.] *J. Mt. Sinai Hosp.*, **10**:172-175. 1943.

While the immediate mortality following radiation treatment of cancer of the cervix is considerably less than that following hysterectomy, the late complications often leading to death are definitely more frequent. The damage done by surgery is immediately evident, whereas the injuries produced by x-rays and radium may take years for development. In group I cancers (League of Nations classification) surgery in patients who are good operative risks presents definite advantages over radiation. In group II cancers, radiation of the primary tumor is advisable because radical surgery gives too high a primary mortality. Radiation can be combined with surgical removal of tributary pelvic lymph nodes, since the primary mortality of this procedure is only 1.6%, and the 5 year additional salvage by this operation is 15%. In group III and group IV cancers, radiation alone should be considered even though it may often be attended by fatal complications and can be expected to save only 1 out of every 5 or 6 patients.—A. Cnl.

Removal of Vulvae and Perineal Body Because of Squamous Cell Epithelioma. Tyler, A. F. [Immanuel Hosp., Omaha, Nebr.] *Am. J. Surg.*, **61**:302-304. 1943.

Report of a case treated surgically and with x-rays (1,600 r to each inguinal region). There was no recurrence 32 months after operation.—W. A. B.

Hematuria Complicating Fibromyoma of the Uterus. Wimpfheimer, S. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:301-304. 1943.

Urologic complications are not uncommonly associated with gynecologic conditions, mostly as a result of pres-

sure on the ureter and bladder by pelvic tumors, or inflammatory disease. In these conditions it is unusual to see hematuria unless some gross lesion is present in the urologic tract. In the case reported, the pressure by the tumor probably produced vascular stasis with rupture of vesical veins resulting in bloody urine. Following the removal of the uterine fibromyomas and adnexa, the ureteral defect made visible preoperatively in the retrograde pyelogram disappeared, as did the hematuria. Cystoscopy at no time revealed any blood from the ureter.—A. Cnl.

Male Genital Tract

Carcinoma of Penis—Report of a Case of Carcinoma and Active Gonorrhea Co-Existing. Caponegro, F. [Greenpoint Hosp., Brooklyn, N. Y.] *Urol. & Cutan. Rev.*, **45**:501-504. 1941.

The etiology of carcinoma of the penis is discussed, and its infrequency after circumcision is stressed. Only a few cases have been reported in circumcised patients. A case is described presenting coexistent gonorrhea, which was treated first. Later, amputation and excision of nodes was performed.—V. F. M.

Testicular Neoplasms (with Case Histories). Fenton, C. C. [Morgantown, W. Va.] *Urol. & Cutan. Rev.*, **45**:681-684. 1941.

The various types of testicular neoplasms are described. A mixed tumor, 3 embryonal carcinomas, and 2 embryonal carcinomas with lymphoid stroma are reported with illustrations.—V. F. M.

Fibroma of the Tunica Vaginalis Testis. Schulte, W. G. [Salt Lake City, Utah] *Northwest Med.*, **41**:60-63. 1942.

Palpable nodules thought to be malignant proved, on removal of the testis, to be multiple fibromas of the tunica vaginalis. Observations concerning all forms of testicular tumors are quoted from the literature.—E. E. S.

Cord Tumor. Whitcomb, B. B. [Hartford Hosp., Hartford, Conn.] *Connecticut M. J.*, **7**:693-696. 1943.

Six cases representing both the typical and the unusual types of cord tumors are reported.—M. E. H.

Perineal Prostatectomy Versus Transurethral Resection for Hypertrophy and Cancer of the Prostate. Young, H. H. [Brady Urological Inst., Johns Hopkins Hosp., Baltimore, Md.] *Surg., Gynec. & Obst.*, **77**:1-15. 1943.

A critical analysis of 200 selected cases indicates that perineal prostatectomy is superior to transurethral resection in the larger hypertrophies, in cases with calculi in the prostate, and in chronic prostatitis. Another great advantage of the perineal procedure is the opportunity it affords to make a diagnosis and effect a cure of carcinoma of the prostate.—J. G. K.

Tumors of the Spermatic Cord: Report of a Hemangioma. Zide, H. H. [Station Hosp., Fort Ord, Calif.] *J. Urol.*, **50**:255-257. 1943.

A hemangioma of the spermatic cord in a 27 year old patient is described.—V. F. M.

Salivary Glands

Primary Malignant Tumors of the Submaxillary Gland with Special Reference to Mixed Tumors. Dockerty, M. B., and Mayo, C. W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:593-603. 1942.

Eighty-one primary submaxillary malignant tumors are reviewed from clinical and pathologic aspects. Adenocarcinoma of the mixed tumor type constituted 63% of the series. They represented low grade, slowly growing adenocarcinomas usually not associated clinically with pain or much local fixation. The tendency toward recurrence was not pronounced except when inadequate tissue had been removed.

Adenocarcinoma of the cylindroma type accounted for 18.5% of the tumors. The cylindromas were as a rule associated with a clinical history somewhat shorter than that of mixed tumors. Pain was a prominent clinical symptom, and local fixation was sometimes observed. Pathologically, the lesions, although they were of a moderate degree of malignancy, presented pronounced infiltrative tendencies with selective invasion of nerves. The rate of recurrence was extremely high, and the outlook generally unfavorable.

An intermediate group of lesions presented the microscopic features of both the mixed tumor and cylindroma but, unfortunately, the prognosis of the latter.

Atypical tumors described as miscellaneous were present in 9.9% of the cases. Prognosis depended partly on the type and grade of the lesion and partly on the extent of involvement of the lymph nodes.

Clinical follow-up studies demonstrated the usefulness of the microscopic classification in relation to prognosis. Disappointing results followed conservative operation on malignant tumors of the submaxillary gland. Tumor and gland should be removed in all cases of mixed tumor. For cylindromas, pure or mixed, and for most of the neoplasms in the miscellaneous group, dissection of the lymph nodes should be carried out as well.—J. L. M.

Gastrointestinal Tract

Clinical and Roentgenologic Manifestations of Tumors of the Small Intestine: Review of Thirty-Five Cases. Good, C. A., and MacCarty, W. C., Jr. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:20-22. 1942.

Roentgenologic examination of the small intestine is difficult and time-consuming. The necessity for prolonged and repeated roentgenoscopic observation increases the amount of radiation received by the patient and the examiner. For this reason it is expedient that patients who are referred for examination be carefully selected and that unnecessary or routine examinations be kept at a minimum.

In order to determine a practical basis for the selection of patients for roentgenologic investigation of the small intestine, a review was made of all cases of tumor of the duodenum, jejunum, or ileum encountered at operation from 1939 to 1941, inclusive. Only those cases were included in which exploration was carried out primarily because of the tumor. Thirty-five cases fulfilled these requirements. The pathologic type and incidence of each lesion are given. By far the most common tumor was adenocarcinoma, with leiomyoma second in frequency. Although carcinoid tumors are considered true adeno-

carcinomas, they are classified separately because of their differing clinical features. The lesions were situated in all segments of the small intestine; 8 occurred in the duodenum, 15 in the jejunum, and 12 in the ileum. Thirteen of the 16 adenocarcinomas, as well as 4 of 6 leiomyomas, were in the upper portion of the small intestine. Study of the records of these patients revealed that the most important clinical manifestations were (a) evidence of loss of blood, (b) the presence of a mass in the abdomen, and (c) evidence of past or present intestinal obstruction. In the majority of instances the lesson can be demonstrated roentgenologically.—J. L. M.

Colonic Polyposis: Its Role in Failure to Effect Cure in Certain Cases of Carcinoma of the Colon and Rectum by Resection of the Primary Lesion. Mayo, C. W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:181-184. 1942.

Records made at necropsy of 334 patients who had died with primary carcinoma of the colon or rectum were studied. It was found that 114 patients, or 34%, had had polyps in addition to the primary neoplasm accounting for the symptoms. In 14% of the cases these polyps were already malignant. In 8 cases there was so-called congenital multiple polyposis; in 31 instances polyps were few but were in multiple situations in the colon, and in 5 cases the situations had not been noted.

Additional study of the series of cases revealed that in 131 resection of the colon or rectum had been performed with the indication at the conclusion of the operation that the patient had a good chance of being cured. Death in this particular group ensued within less than 3 weeks after operation. Therefore, it can be assumed that no change relative to polyps of the colon would have taken place in the short time between operation and death. It was found that in 49, or 37%, of these latter cases, there were remaining polyps.

In order better to interpret these observations, records made at necropsy of a control series of 100 patients who had died after surgical operations performed for conditions other than malignancy were studied. It was found that in 16 instances polyps of the colon or rectum were present, and in 8 of these the polyps were malignant.—J. L. M.

Carcinomatous Sigmoidovesical Fistula Associated with Multiple Polypoid Disease of the Colon: Report of Case. Mayo, C. W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:460-461. 1942.

This case is reported because it demonstrates that not all sigmoidovesical fistulas caused by malignant lesions are inoperable, that multiple polypoid disease of the colon is a serious problem requiring radical surgical procedures, but that in selected cases the rectum can be preserved and an abdominal ileal stoma obviated.—J. L. M.

Lymphosarcoma of the Intestine. Report of Two Cases. Menne, F. R., Mason, D. G., and Johnston, R. [Univ. of Oregon Med. Sch., Portland, Ore., and St. Luke's Hosp., San Francisco, Calif.] *Arch. Surg.*, **45**:945-956. 1942.

A brief review of the literature and report of 2 cases. Both lesions, which were removed at operation, involved the terminal ileum and cecum. One patient shows no evidence of recurrence 4 years after operation; the other died of recurrence of the tumor.—G. H. H.

Premalignant Lesions of Colon, Rectum, and Anus. Parkinson, E. D. [Boise, Idaho] *Northwest Med.*, **41**: 206-208. 1942.

Polypi occurring in the distal portion of the colon are considered important lesions because of the danger of malignant transformation. In the anal region the precancerous lesions are said to be fistulae, ulcers, hemorrhoids, papillitis, and pruritus. Papillomas with a broad base are especially likely to become malignant. Excess mucous production or hemorrhage may lead to their detection. Pain is seldom present but lesions in the lower rectum may produce tenesmus. It is believed that at least 14% of persons have colonic or rectal polypi. Removal of polypi is urged as a prophylactic measure against cancer. —E. E. S.

Transthoracic Resection of the Stomach and Esophagus for Carcinoma: Report of Case. Walters, W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:241-247. 1942.

In this paper the author reports the first case in which transthoracic resection of the stomach was performed successfully at the Mayo Clinic and considers briefly some results obtained by, and the value of, the clinical investigation of surgical problems.—J. L. M.

Surgery of the Terminal Ileum, Cecum, and Right Colon. Whipple, A. O. [Columbia-Presbyterian Med. Center, New York, N. Y.] *Surgery*, **14**:321-327. 1943.

General discussion with presentation of immediate results of various procedures for cancer and other diseases.—W. A. B.

Cancer of the Colon. Zinninger, M. M., and Hoxworth, P. I. [Univ. of Cincinnati, and Cincinnati Gen. Hosp., Cincinnati, Ohio] *Surgery*, **14**:366-377. 1943.

The technic of resection and anastamosis for cancer of the colon is described. The mortality among 45 patients operated upon was 8.8%.—W. A. B.

Liver

Primary Carcinoma of the Gallbladder. Report of Two Cases. Berghausen, O. [Cincinnati, Ohio] *Ohio State M. J.* **38**:125-127. 1942.

The lowered incidence of this tumor in the past 25 years is noted. There is a brief summary of the clinical course and a list of the autopsy findings in 2 patients having carcinoma of the gall bladder. A history of biliary colic was not elicited from either patient; one had cholelithiasis with suppurative infection of the gall bladder. Three patients with secondary carcinoma in the liver are also described.—E. E. S.

Benign Neoplasms of the Gallbladder. Shepard, V. D., Walters, W., and Dockerty, M. B. [Mayo Clinic, Rochester, Minn.] *Arch. Surg.*, **45**:1-18. 1942.

A review of the literature and a study of 150 cases of benign tumors of the gallbladder. The types of tumor encountered are polypus, adenomyoma, and fibroma. Preoperative diagnosis can be made only by means of cholecystography. Chronic cholecystitis is always found in association with these tumors, and cholelithiasis is usually present. The incidence of malignant change is low. —G. H. H.

Carcinoma of the Biliary Papilla. A Case Record Presenting Clinical Problems. Webb, J. P., and Johns-

MANN, R. [Cincinnati Gen. Hosp., Cincinnati, Ohio] *Ohio State M. J.*, **38**:763. 1942.

The paper records the detailed history.—E. E. S.

Spleen

Malignant Neoplasms of the Spleen. Review of the Literature and Report of a Case of Primary Lymphosarcoma (Reticulum-Cell Type). HAUSMANN, P. F., and GAARDE, F. W. [Mayo Clinic, Rochester, Minn.] *Surgery*, **14**:246-255. 1943.

Splenectomy was performed for a "primary reticulum-cell lymphosarcoma" of the spleen. The patient, aged 38, received x-ray therapy postoperatively but died approximately 1½ years after the onset of symptoms and 10 months after operation without generalized involvement of lymph nodes.

A review of malignant splenic neoplasms is presented. Lymphosarcoma is the commonest type. Generalized lymphosarcomatosis subsequently developed in only 2 of 9 cases of splenic lymphosarcoma reported in the literature and in 1 of 4 cases followed at the Mayo Clinic. Although the number of cases of lymphosarcoma studied was small, the authors believe that splenectomy is justifiable for lymphosarcoma confined to the spleen.—W. A. B.

Adrenal

The Estrogenic Reaction in Adrenal Cortical Carcinoma. FRANK, R. T. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **8**:514-519. 1942.

In 1934 attention was drawn to the great excess of estrogen excretion in 2 patients with adrenal cortical carcinoma. Since then 6 more patients have been under observation. Assays for estrogen were made by the injection of fresh urine into castrated mice. A positive vaginal smear with 1 cc. of urine indicates a minimum of 1000 I.U. per liter. A positive reaction has been obtained from as low a urine titer as 0.075 (13,000 I.U. estrogen/L). Negative tests were obtained uniformly in the many controls covering a great variety of conditions including hirsutism, obesity, and high blood pressure. In female patients a negative pregnancy test is required, as there is a physiologic increase in estrogen excretion during the gravid state.—S. A. G.

Surgical Treatment of Lesions of the Adrenal Glands. WALTERS, W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:223-224. 1942.

The treatment of tumors of the adrenal glands is, of course, surgical removal of the tumor. Although transthoracic, transpleural, and retroperitoneal approaches to the adrenal glands have been used, in the opinion of the author the approach that gives the best opportunity for minute and careful inspection of the gland is a posterolumbar incision similar to that used in exposing the kidney. After the fascia of Gerota has been incised, the perirenal fat is reflected from the upper pole of the kidney, and the kidney is retracted downward; this exposes the inferior and posterior aspects of the adrenal gland. It is possible in this manner to study the gland accurately from every side without disturbing the circulation.

At the Mayo Clinic, 9 hyperfunctioning cortical tumors and 3 medullary tumors have been removed without any mortality. Two of the cortical tumors were very large and weighed 600 and 1,050 gm. respectively, whereas the others averaged approximately 10 to 15 gm. and were 2 to 4 cm. in diameter. In addition, in 3 cases malignant adrenal tumors that had not produced hyperfunction were removed without mortality.

Although microscopic examination revealed early malignant changes in all of the cortical tumors, in most of the cases the tumor was encapsulated definitely, and recurrence has not taken place. In the 2 cases in which the cortical tumors were very large, the tumor had penetrated the capsule: the growth was attached to the diaphragm in one case and to the inferior vena cava in the other. In both cases the lesions were malignant. There were signs and symptoms of recurrence, and both patients died within 2 years after operation. The 3 medullary tumors that were removed were encapsulated and benign.—J. L. M.

Miscellaneous

Tumor Diagnostic Services in Illinois. OTTEN, H. [Springfield, Ill.] *Illinois M. J.*, **84**:312-317. 1943.

The author seeks to develop greater interest in early diagnosis and treatment of all tumors, and to increase the facilities offered, as well as the scope of the work, by a presentation of the tumor diagnostic services available in Illinois. These services were initiated in Illinois in 1940.—M. E. H.

Minimal Criteria Required to Prove Causation of Traumatic or Occupational Neoplasms. WARREN, S. [Harvard Cancer Comm., New England Deaconess Hosp. and Harvard Med. Sch., Boston, Mass.] *Ann. Surg.*, **117**:585-595. 1943.

To prove trauma responsible for a tumor, at least the continuity of the previously unbroken tissue concerned must be interrupted with regenerative processes resulting, the neoplasm must follow after a reasonable time interval, and the type of atypical growth must be reasonable for the site of injury. The most convincing evidence for the occupational nature of tumors is the significantly greater incidence of a tumor, characteristic in type and location, among individuals occupied in a single industry, when compared to similar data for the general population. When application of the agent leads to the appearance of tumors in animals, the conclusion has additional support. The author gives a modification of Hueper's classification of occupational neoplasms.—W. J. B.